RESPONSES TO GAMMA-AMINOBUTYRIC ACID OF RAT VISUAL CORTICAL NEURONS IN TISSUE SLICES

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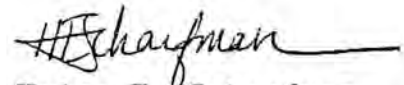

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ABSTRACT

Title of Dissertation: Responses to Gamma-Aminobutyric Acid of Rat Visual Cortical Neurons in Tissue Slices

Helen Edith Scharfman, Doctor of Philosophy, 1986

Dissertation directed by: John M. Sarvey, Assistant Professor, Department of Pharmacology

This project was undertaken to establish the effects of gamma-aminobutyric acid (GABA) on individual visual cortical neurons. Intracellular recordings were made from 148 Layer V neurons using slices of rat visual cortex. Slices (400 um thick) were warmed, oxygenated and perfused with artificial cerebrospinal fluid. GABA was ejected from a micropipette onto the soma and dendrites (n=87). Intracellular staining techniques were used to identify 20 of these cells morphologically.

Pyramidal and nonpyramidal cells could be differentiated on the basis of their responses to afferent stimulation, but they did not appear to differ in their responses to intracellular current injection. Nor did they differ in their responses to GABA. All neurons tested responded to GABA ejected within 300 um of the soma, and three types of GABA responses were found. They were often elicited in combination, and were accompanied by increases in membrane conductance: (1) the $GABA_s$ response, elicited by somatic application of GABA (mean reversal potential $\pm$ s.e.m.=-71.7 $\pm$ 1.1 mV), (2) the $GABA_d$ response, elicited by dendritic ejection of GABA (reversal potential=-49.3 $\pm$ 2.4 mV), and (3) a prolonged late hyperpolarization, which could be elicited by somatic or dendritic application, and followed $GABA_s$ or $GABA_d$ responses (reversal potential=-79.8 $\pm$ 1.7 mV).

The $GABA_A$ receptor antagonist bicuculline methiodide (0.1-50 uM) simultaneously depressed GABA responses and invoked epileptiform activity. Pentobarbital (500 uM-1 mM) and diazepam (1 uM-500 uM), potentiated GABA responses. These data support the hypothesis that $GABA_s$ and $GABA_d$ responses are mediated by $GABA_A$ receptors. Similarities between the response to pressure-application of the $GABA_B$ receptor agonist baclofen (1 uM-1 mM) and the late hyperpolarization indicated that $GABA_B$ receptor activation may be responsible for the late hyperpolarization. Other experiments (n=16) indicated that chloride and potassium ions underly $GABA_s$ and $GABA_d$ responses, whereas calcium and sodium do not appear to be involved. The late hyperpolarization appears to be entirely potassium-dependent.

The similarity of the $GABA_s$ response and the late hyperpolarization to the early and late phases of the inhibitory postsynaptic potential (IPSP), respectively, support the hypothesis that GABA is the transmitter which mediates the early and late phases of the IPSP in visual cortical cells. This work provides insight into the mechanisms of action of GABA and emphasizes the importance of this compound to visual cortical function.

RESPONSES TO GAMMA-AMINOBUTYRIC ACID OF RAT VISUAL CORTICAL NEURONS RECORDED IN TISSUE SLICES

by

Helen Edith Scharfman

Dissertation submitted to the Faculty of the Department of Pharmacology
Graduate Program of the Uniformed Services University of the
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INTRODUCTION

HISTORY AND BACKGROUND

Roberts and Awapara were the first to isolate GABA from brain tissue, independently, in 1950 (Awapara, 1950; Roberts and Frankel, 1950). At that time it was thought to be merely a metabolite. The first suggestion that its actions were inhibitory came from Florey (Florey, 1953). He found that an extract of mammalian brain, which he named Factor I, inhibited discharge of the abdominal stretch receptor neuron in crayfish. Factor I was later identified as GABA (Bazemore et al., 1957). The next breakthrough came in 1958, when Kuffler and Edwards found that GABA mimicked the effects of afferent stimulation of the crayfish stretch receptor neuron, and suggested that GABA might be the transmitter at this synapse (Kuffler and Edwards, 1958).

This early work in crayfish prompted investigation of the action of GABA in mammalian brain, where it is found in high concentrations in almost all areas (Young et al., 1976; McGeer et al., 1978). GABA is not necessarily confined to discrete pathways, as is the case for other classical transmitters such as acetylcholine or norepinephrine. Rather, it is typically found in inhibitory interneurons, which act as local circuit neurons, controlling the activity of other neurons in the same area of the brain (Rakic, 1975; Ito, 1976; Shepherd, 1979).

Considering the widespread distribution of GABA and the high levels in the brain, it not surprising that problems concerning GABAergic transmission have been linked to several different diseases of the CNS. One of the best examples is epilepsy, which is hypothesized to result from a loss of GABA-mediated inhibition, producing a state of hyperexcitablity and eventually seizures (Fariello et al., 1984). Several

movement disorders are also related to a loss of GABAergic function. Huntington's chorea, for example, is associated with degeneration of GABAergic efferents arising from the corpus striatum. GABA has also been linked to Parkinson's disease, olivo-vestibular disorders, and spasticity (Enna, 1981; Morselli and Lloyd, 1983). Neuropsychiatric illnesses, such as schizophrenia and depression, as well as regulation of anxiety and aggressive behavior, may be dependent on proper GABA function in the limbic system (DeFeudis and Orensanz-Munoz, 1980; Krogsgaard-Larsen, 1981; Morselli and Lloyd, 1983). In addition, there is evidence that GABA plays a role in analgesia (Hill et al., 1981), and it may have endocrine and cardiovascular functions as well (Muller et al., 1983). GABA has even been proposed as a trophic factor which regulates synaptogenesis early in development (Chronwall and Wolff, 1978).

SYNTHESIS, RELEASE, AND METABOLISM

GABA is the decarboxylated form of the amino acid glutamate. In GABAergic neurons glutamate is converted to GABA by the action of glutamic-acid decarboxylase (GAD), which requires pyridoxal phosphate as a cofactor and is the rate limiting enzyme for GABA synthesis (McGeer et al., 1978; Haefly et al., 1983). Upon electrical stimulation or depolarization by elevated concentrations of potassium, GABA is released into the synaptic cleft by a calcium-dependent process (Iversen et al., 1971; Ryan and Roskoski, 1975). GABA binds to its receptor which, in most cases, leads to inhibition of the activity of the postsynaptic neurons. Sodium-dependent presynaptic uptake (Iversen and Neal, 1968; Storm-Mathisen et al., 1976) and uptake of GABA by glia (Iversen and Kelly, 1975; Hamberger et al., 1981) play an important role in terminating the actions of GABA, as does diffusion (Dingledine and Korn,

1986). GABA-transaminase, located primarily in mitochondria, metabolizes GABA to succinic acid semialdehyde, which can be converted to succinic acid and used in the Krebs Cycle (McGeer et al., 1978; Haefly et al., 1983).

GABA RECEPTORS

It is agreed that more than one GABA receptor exists, although that is where the agreement ends. Separation of the subtypes of GABA receptor have been argued to be based on: pre vs. postsynaptic, synaptic vs. extrasynaptic, or somatic vs. dendritic location (Simmonds, 1984). There may be one receptor subtype that binds GABA in its extended conformation, and another that binds GABA in its folded conformation (Nistri and Constanti, 1979). Another possible segregation is based on whether the receptor is or is not linked to a site where benzodiazepines bind (Olsen, 1981). The most common point of view is that there is a $GABA_A$ and a $GABA_B$ receptor, based on different pharmacological sensitivity to agonists and antagonists (Simmonds, 1984).

Much more is known about the $GABA_A$ receptor than the $GABA_B$ receptor. The $GABA_A$ receptor is a complex of three binding sites that surround a chloride channel. GABA and its agonists and competitive antagonists (bicuculline) bind to the GABA recognition site. (Krogsgaard-Larsen, 1981; Olsen, 1981; Gallagher and Shinnick-Gallagher, 1983). Benzodiazepines bind to a second site and are thought to enhance GABA binding by an allosteric mechanism (Costa and Guidotti, 1979; Olsen, 1981; Toffano, 1983). This class of drugs is thought to displace an endogenous inhibitor of GABA binding, which normally occupies the benzodiazepine binding site (Costa and Guidotti, 1979; Toffano, 1983). The barbiturates also enhance the binding of GABA. This class of drugs

are thought to bind to a third site, as well as directly effecting the chloride channel (Nicoll et al., 1978, Olsen, 1981).

Recently, evidence has accumulated for a second receptor subtype, the $GABA_B$ receptor. The presence of this receptor was initially shown in rat sympathetic ganglia, where it was demonstrated that GABA acted presynaptically to decrease transmitter release as well as postsynaptically to depolarize neurons. (Bowery et al., 1980 and 1981). Subsequently it was found that GABA decreased transmitter release in other peripheral and some central preparations, such as chick sensory ganglia (Dunlap and Fischbach, 1981), guinea pig cortical slices (Potashner, 1979), rat hippocampus (Ault and Nadler, 1980, Lanthorn and Cotman, 1981), and rat whole brain (Hill and Bowery, 1981). However, it has recently been shown in hippocampus and substantia nigra that the $GABA_B$ receptor agonist B-(p-chlorophenyl)-GABA (baclofen) has a direct postsynaptic action (Newberry and Nicoll, 1984a and b; Gahwiler and Brown, 1985; Inoue et al., 1985; Newberry and Nicoll, 1985), so the effects of $GABA_B$ receptor activation are not entirely clear.

The main discriminating feature between the $GABA_A$ and $GABA_B$ receptor is their sensitivity to bicuculline. $GABA_A$ receptors are bicuculline-sensitive, whereas $GABA_B$ receptors are not. There are only two known agonists for the $GABA_B$ receptors: GABA and baclofen; no known antagonist exists. In contrast to the $GABA_A$ receptor, benzodiazepines do not enhance the binding of GABA to the $GABA_B$ receptor. Furthermore, $GABA_B$ binding requires divalent cations, such as calcium and magnesium, and decreases in the presence of GTP (Bowery et al., 1983). It is possible that baclofen acts through the second messenger cAMP (Bowery et al., 1983; Wojcik and Neff, 1983; Dolphin, 1984).

GABA-MEDIATED INHIBITION

The binding of GABA to the $GABA_A$ receptor has been reported to open a chloride channel (Olsen, 1981). Chloride ions pass into or out of the cell, following their electrochemical gradient. Either a hyperpolarization or a depolarization is produced by the movement of chloride ions, and an increase in conductance is observed (Allen et al., 1977, Eccles et al., 1977).

Much less is known about the $GABA_B$ receptor. It may be associated with a chloride channel or, as some have proposed, a calcium channel (Dolphin, 1984). $GABA_B$ receptor stimulation may decrease transmitter release by decreasing calcium influx into presynaptic nerve terminals (Dunlap and Fischbach, 1981, Konnerth and Heinemann, 1983). The postsynaptic actions of baclofen could also be attributed to a calcium channel (Dolphin, 1984); the sensitivity of $GABA_B$ receptor binding to GTP has led some to propose that the calcium channel is linked to adenylate cyclase (Bowery et al., 1983). However others suggest that potassium ions may be involved in the postsynaptic effect (Newberry and Nicoll, 1984a and b; Gahwiler and Brown, 1985; Inoue et al., 1985; Newberry and Nicoll, 1985).

GABA IN VISUAL CORTEX

The visual cortex of the rat is composed of six layers that extend from the pia to the white matter. Pyramidal cells are located in layers II/III, V, and VI, whereas nonpyramidal cells are present in all layers. It has been shown that most afferents synapse in layer IV, either on the cell bodies of nonpyramidal stellate cells or on the dendrites of pyramidal cells whose cell bodies are located in layers III

and V. The stellate cells project to the pyramidal cells in layers V and VI, as well as the smaller pyramidal cells of layers II/III, and many pyramidal cells send recurrent collaterals throughout the cortical layers. The pyramidal cells form the efferent systems which project to other areas of the brain. This seemingly simple circuit is made immensely complex by the presence of many more nonpyramidal cell types, which make synaptic connections between the axon collaterals and dendritic processes of both the stellate and pyramidal neurons (de No, 1949; Creutzfeldt, 1977; Szentagothai, 1973; Parnavelas et al., 1977; Shepherd, 1979; Peters 1981; White, 1981; Evarts et al., 1984; Jones, 1984; Peters and Kara, 1985a and b, Figure 1).

Very little is known about GABA in the visual cortex. However, several studies have provided strong indications that it is an inhibitory neurotransmitter released from interneurons onto pyramidal cells an nonpyramidal cells. Many types of interneurons in all layers of the cortex are labelled by ^{3}H-GABA (Somogyi et al., 1984a; Wolff and Chronwall, 1982). Other studies have shown the widespread distribution of GABAergic cells using antibodies to GABA (Ottersen and Storm-Mathisen, 1984) or GAD (Ribak, 1978; Peters et al., 1982; Freund et al., 1983). A single population of binding sites in cat visual cortex has been labelled with the GABA agonist muscimol (Needler et al., 1984). Specific binding has also been reported for GABA and baclofen in all areas of the cerebral cortex (Bowery et al., 1983; Enna and Gallagher, 1983).

There are several studies that link GABAergic nonpyramidal cells and GABA release with inhibition in visual cortex, which adds support to the hypothesis that GABA is an inhibitory transmitter. For example, it has been shown that GABAergic nerve terminals make symmetric, type II synapses (Somogyi et al., 1982; Wolff and Chronwall, 1982; Freund et al.,

1983) which are thought to be inhibitory (Gray, 1959; Colonnier, 1968). Inhibition in cat visual cortex is associated with the release of GABA above unstimulated levels (Iversen et al., 1971). The GABA antagonist bicuculline has been shown to increase evoked and spontaneous unitary activity in cat visual cortex (Sillito, 1975a and b). It also dramatically alters receptive field properties and the activity of simple and complex cells (Pettigrew and Daniels 1973; Rose and Blakemore, 1974; Sillito, 1975a and b), many of which may be pyramidal cells (Kelly and Van Essen, 1977).

PROPOSED RESEARCH APPROACH

These studies suggest that GABA is an inhibitory transmitter of visual cortical interneurons, and that it has an important role in the control of visual cortical activity. However, in spite of its importance, very little is known about the actions of GABA at the single cell level. Previous studies have only recorded extracellular responses to GABA, which do not provide resolution at the single cell level.

Thus, the primary goal of the present study was to provide an intracellular analysis of the effects of GABA on visual cortical cells. Of particular interest were the actions of GABA on pyramidal cells, because of (1) the strong evidence relating GABAergic interneurons to inhibition of visual cortical cells (see above), and (2) the central role of pyramidal cells in cortical processing and efferent activity (Szentagothai, 1973; Creutzfeldt, 1977; White, 1981; Evarts et al., 1985). Also of interest were the differences in the physiology of morphologically distinct cells: the pyramidal and nonpyramidal cells.

There were several methodological obstacles to overcome. First, it was necessary to develop a preparation for intracellular recording in

the visual cortex. In vivo recording was undesirable because it is plagued by problems which make stable recording extremely difficult, such as movements caused by respiration. In addition, there is limited access to the brain for stimulation, recording, or applying drugs to discrete areas. In other areas of the CNS, slice preparations have been adopted to circumvent these problems (Schwartzkroin, 1981). Thus, it was anticipated that visual cortical slices might facilitate intracellular recording in the visual cortex and afford the opportunity to examine the actions of GABA at the single cell level.

Since the intracellular characteristics of visual cortical cells were unknown, another important first step was obtaining a preliminary description of the activity of these cells, especially layer V. Identification of responses to synaptic stimulation and current injection would provide an indication of the types of activity that visual cortical neurons are capable of, and those that GABA might affect. In particular, subthreshold synaptic responses were to be examined, because, by comparing synaptic responses to GABA responses, the role of GABA as a neurotransmitter might be clarified.

Next, the central question concerning how GABA affects Layer V neurons could be approached. GABA was applied focally to many different cells, and to different areas of the same cell. To pursue the actions of GABA further, the effects of pharmacological and ionic manipulations of the perfusing medium could be tested.

To determine the responses of pyramidal cells in particular, two steps were necessary. First, recording was restricted to Layer V, which contains the greatest density of pyramidal cells. Second, cells were morphologically identified by intracellular dye injection during the electrophysiological recording procedure.

SPECIFIC AIMS

In summary, the specific aims of the project were:

1. Physiological characterization of the neurons in Layer V of rat visual cortical slices by intracellular recording.
    a. development of a visual cortical slice preparation.
    b. characterization of responses to orthodromic stimulation and current injection.
2. Definition of the responses to GABA of Layer V neurons, their pharmacology, and possible ionic mechanisms.
    a. identify responses to GABA ejection on the soma and dendrites
    b. determine effects of several GABAergic agents on the GABA responses: bicuculline, pentobarbital, diazepam, and baclofen.
    c. determine which ions mediate GABA responses (i.e. chloride, potassium, calcium, or sodium).
    d. obtain data to address the hypothesis that GABA is a neurotransmitter in the visual cortex
3. Histological identification of cells after characterization of physiological and GABA responses by intracellular staining techniques to identify them as pyramidal or nonpyramidal.

MATERIALS AND METHODS

DEFINITION OF VISUAL CORTEX

The boundaries of the rat visual cortex (areas 17 and 18) were defined on the basis of several sources (Krieg, 1946a, 1946b, Adams and Forrester, 1968, Zilles et al., 1980, Parnavelas et al., 1977; Peters 1981; Zilles, 1985). The definitions of cortical layers were based on the work of others (Parnavelas et al., 1977; Peters, 1981). Layer I extends from 0-150 um from the pial edge of the slice. It is accepted that the border of Layer II with Layer III is extremely difficult to distinguish in rat visual cortex (Szentagothai, 1973, Creutzfeldt 1977, Parnavelas et al., 1977a, 1977b, Peters, 1981), and therefore Layers II and III are grouped together. Layers II/III extend from 150-525 um from the pia. The boundaries of Layer IV are 525 and 750 um fom the pia, those of Layer V are 750 and 1250 um, and Layer VI stretches from 1250-1500 um from the pia (Figure 1D).

PREPARATION OF VISUAL CORTICAL SLICES

Adult, male, Sprague-Dawley rats (150-300g) were obtained from Hilltop Lab Animals (Scottsdale, PA). They were given free access to food (M/R/H 2000, Agway, Inc., NY) and water, and were kept on a 12 hour light/ 12 hour dark schedule.

Animals were decapitated and the brain anterior to the cerebellum was removed. The hemispheres were separated with one cut along the midline with a stainless steel razor blade. The occipital cortex of each hemisphere was separated by a coronal section 5 mm from the caudal edge of each hemisphere, and most of the underlying subcortical tissue was removed (Figure 1). The medial areas of these pieces of tissue were the

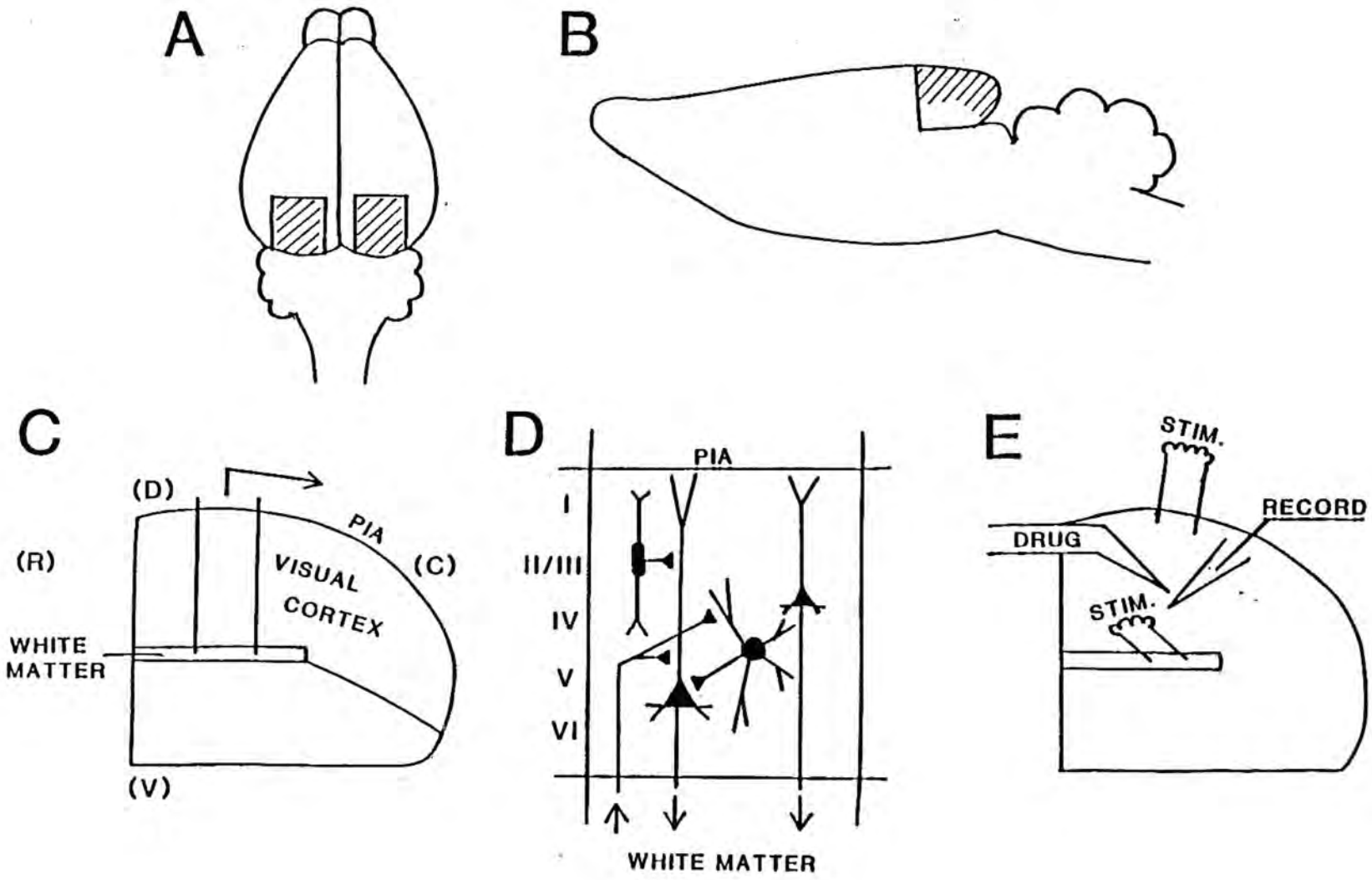
Figure 1. Preparation and recording from visual cortical slices.

A. and B. The visual cortex of the rat (shaded regions) is located in the dorso-caudal area of the cerebral hemispheres.

C. A diagram of a typical visual cortical slice. D= dorsal, V= ventral, R=rostral, C=caudal.

D. A portion of the visual cortical slice shown in C is enlarged to show the orientation of some of the neurons. Triangle=pyramidal cell, circle=multipolar, oval=bipolar.

E. A diagram of the visual cortical slice which illustrates the electrophysiological recording arrangement. The recording electrode was usually placed in Layer V. Bipolar electrodes were used to stimulate the pial surface (PIA) or the white matter (WM). A micropipette filled with GABA (10 mM) was placed 0-500 um from the intracellular recording electrode for pressure application of GABA.


A
B
C
(D)
PIA
(R)
VISUAL
CORTEX
(C)
WHITE
MATTER
(V)
D
PIA
I
II/III
IV
V
VI
WHITE MATTER
E
STIM.
RECORD
DRUG
STIM.

removed (Figure 1). The medial areas of these pieces of tissue were the visual cortices.

Each visual cortex was sliced into 400-450 um thick sagittal sections with a mechanical chopper (Brinkmann Instruments Inc., Westbury, NY). Slices were removed from the blade of the chopper with a paintbrush and were immediately placed in a small beaker that contained warmed ($30^{o}C$), oxygenated (95% O_2, 5% CO_2), modified Krebs-Ringer solution (in mM: 124 sodium chloride, 3 potassium chloride, 1.3 magnesium sulfate, 1.25 sodium phosphate (monobasic), 2.4 calcium chloride, 26 sodium bicarbonate, 10 glucose; pH= 7.2-7.4). Chemicals were obtained from Fisher Scientific Co., Fair Lawn, NJ. The buffer was warmed by placing the beaker in a Haake waterbath (Fisher). Oxygenation was supplied through 1 ml pipettes placed in the beakers. The dissection and slicing procedure was completed in 10 minutes.

After 45-60 minutes 2-3 slices were transferred to an interface recording chamber (Schwartzkroin, 1975), where they were allowed to equilibrate one hour. When additional slices were used later in the same day (up to 12 hours post-dissection), they were also allowed to equilibrate in the recording chamber for one hour. There were no differences in the recordings that could be attributed to the amount of time that had passed since dissection.

Slices were supported on a nylon mesh (500 um sq., Small Parts Inc., Miami, FL) in the inner compartment of the chamber, which was constructed at NIH (Bethesda, MD). Slices were perfused with warmed, oxygenated, modified Krebs-Ringer solution at a rate of 2 ml/minute using a peristaltic pump (Minipuls 2, Gilson Medical Electronics, Middleton, WI). The solution was warmed to $37^{o}C$ in a GCA Precision Scientific waterbath (Chicago, IL), and oxygenated prior to entry into the chamber.

The inner compartment was surrounded by an outer compartment that contained warmed (35°C) and oxygenated (95% O_2, 5% CO_2) water. The heater for the outer compartment was made at the NIH. Humidified oxygen flowed over the slice by passing through vents from the outer compartment to the inner compartment. 95% O_2, 5% CO_2 was supplied to the outer compartment at a rate of 200-250 ml/minute as monitored by a gas flowmeter (Gilmont Instruments, Great Neck, NY).

INTRACELLULAR STAINING

Morphological identification of visual cortical neurons was made following intracellular injection of the fluorescent dye Lucifer yellow (Stewart, 1978; Knowles et al., 1982). Recording electrodes used for intracellular dye injection were first filled with potassium methylsulphate and then screened for ability to pass current and impale neurons. Acceptable electrodes were then refilled with a 1-3% solution of the lithium salt of lucifer yellow CH (in deionized water; Sigma). A half cell (World Precison Instruments, New Haven, CT) filled with 1 M lithium chloride (Sigma) was used between the electrode and the preamplifier probe. Several approaches were used to eject dye while maintaining high quality impalements for several hours. Hyperpolarizing DC current (0.25-1.5 nA), current pulses ($\pm$ 0.5-1 nA, 0.5-1.5 sec at 0.33-1 Hz), or both hyperpolarizing DC and hyperpolarizing pulses were passed through the recording electrode, for 30 sec to 10 min periods during the course of the experiment.

After the experiment the intracellular electrode was slowly withdrawn from the slice, and the slice was placed in 10% buffered neutral formalin and refrigerated at 4°C for 3 hours to 2 days. The formalin solution was made by adding 100 ml 37% formaldehyde (J.T. Baker

Chemical Co., Phildelphia, PA), 4.0g sodium bicarbonate, and 6.5g sodium phosphate (diphasic; Fisher) to 900 ml deionized water. In preparation for photography, each slice was dehydrated in ethanol (70% for 3 minutes, 100% for 15 minutes), and cleared in methyl salicylate (approximately 5 minutes, Sigma). These methods are modifications of those used by W. Stewart, who originally developed the technique (Stewart, 1978), and the protocols of others who have used it to stain pyramidal cells in hippocampal slices (Knowles et al., 1982).

Cells were photographed with a Nikon Fluophot Research Fluorescence Microscope and a Nikon Automatic Microflex Photomicrographic System (Nikon, Garden City, NY) using a violet excitation light (maximum wavelength 410-420 nm) and an absorption filter which transmitted wavelengths over 450 nm. Ektachrome 400 or 800/1600 ASA color slide film (Eastman Kodak Co., Rochester, NY) was used.

CRITERIA USED TO CLASSIFY CELLS

A cell was identified as pyramidal if it fulfilled the following criteria (Szentagothai, 1973, Parnavelas, 1977; Peters, 1981; Jones, 1984; Peters and Kara, 1985a):

1. A soma located in layer II/III, V, or VI.
2. A soma with a pyramidal shape, with the apex directed at the pial surface.
3. One large apical dendrite arising from the apex of the soma, and extending towards the pial surface.
4. Basal dendritic trees arising from the base of the soma.
5. The presence of dendritic spines.

There have been several different classifications made of nonpyramidal cells of rat visual cortex, based on (1) shape of the cell

body, (2) general branching pattern of dendrites and axon, and (3) relative number of dendritic spines (Parnavelas et al., 1977, Feldman and Peters, 1978, Peters and Kara, 1985b). In this study it was not possible to differentiate the axon of nonpyramidal cells, nor to use electron microscopy, so cells were classified at the light microscopic level by the criteria of Feldman and Peters (1978). Cells were classified as multipolar, bitufted, or bipolar, and either spiny, sparsely spiny, or spine-free (Feldman and Peters, 1978). The following criteria defined nonpyramidal cells (Feldman and Peters, 1978):

1. A soma located in any layer.
2. A soma that was circular, oval, or irregular.
3. Dendrites radiating from all areas of the soma (multipolar cells) or from two poles (bipolar or bitufted cells).

ELECTROPHYSIOLOGICAL RECORDING

Bipolar stimulating electrodes were made from twisted, Teflon-coated, stainless steel wire (0.1 mm diameter; Cooner Wire Co., Chatsworth, CA). Stimuli (10-500 uA, 50-250 usec square pulses) were generated from 1850A DC stimulus isolation units (WPI) and were triggered by a 1830 WPI interval generator. Calibration pulses preceding each stimulus were supplied by a pulse from a Stoelting calibrator (Stoelting Co., Chicago, IL). Stimuli were delivered to the curved edge of the slice where the pia mater covers the cortex in vivo (PIA), and to the white matter (WM) directly below layer VI (Figure 1C-E). Stimulus frequency was 0.33 Hz while impaling neurons, and 0.1 Hz while recording from an impaled cell. Stimulation of PIA activates pyramidal cell dendrites and nonpyramidal cell processes. Stimulation of WM activates afferents, such as those from the lateral geniculate nucleus, as well as pyramidal cell

axons (Szentagothai, 1973; Parnavelas et al., 1977; Peters, 1981; White, 1981; Jones, 1984).

Recording electrodes were pulled horizontally on a Brown-Flaming Micropipette Puller (Sutter Instruments, San Francisco, CA) using borosilicate glass with a capillary fiber in the lumen (1.2 mm outer diameter, 0.6 mm inner diameter; Frederick Haer Co., Brunswick, ME). Electrodes were filled with 1 M filtered potassium methylsulphate (60-200 megohms; Pfaltz and Bauer Inc., Waterbury, CT) or 3M potassium chloride (40-100 megohms; Sigma Chemical Co., St. Louis, MO). A high impedance amplifier (WPI S7071) with a bridge circuit was used for intracellular recording, and the bridge was balanced when current was passed from the electrode. A silver wire coated with silver chloride was inserted in the perfusing medium under the nylon net holding the slices to provide a ground.

A fiber optics light was used to illuminate the chamber from above (Mathematical Associates, Great, Neck, NY). Cell activity was monitored audibly by a Grass AM7 Audio Monitor (Grass Medical Instruments, Quincy, MA). A Zeiss operating microscope Model OPM1 (Zeiss Inc., Oberkochen, West Germany) was used to visualize the slices for placement of electrodes. An ocular micrometer in the eyepiece was used to measure the thickness of the cortex, as well as the distances between the intracellular electrode, pressure pipette, and WM or PIA. Electrodes were held by micromanipulators (Prior, England, and Leitz Inc., Rockleigh, NJ). The recording chamber and the manipulators rested on a vibration-free air table (Vibraplane, Kinetic Systems Inc., Boston, MA).

A chart recorder (Gould Inc., Cleveland, OH) and a Tektronix 7D20 digitizing plug-in in a Tektronix R7704 Oscilloscope mainframe oscilloscope (sampling rate, 10-50 kHz; Tektronix, Beaverton, OR) were

used to record data. Digitized data were transfered by an IEEE 488 Instrumentation Bus (1980 standard, International Society of Electrical Engineers) to a MINC 11/23 laboratory computer (Digital Equipment Corp., Marlboro, MA), and were saved on floppy disks (Federal Sales Service Inc., Alexandria, VA) for later analysis.

Membrane potential, input resistance, synaptic responses, and responses to GABA or (-)-baclofen were measured from the chart recordings. Action potential amplitude was measured directly from the oscilloscope. Digitized data were plotted on a Hewlett-Packard 7225A plotter (Hewlett-Packard Co., Fullerton, CA).

TERMINOLOGY

The depolarizations following orthodromic or antidromic action potentials were referred to as depolarizing afterpotentials (DAPs; Figure 3). The afterhyperpolarization (AHP) was defined as the hyperpolarization that follow one or more orthodromic action potentials or action potentials elicited during a depolarizing current pulse (Figure 3). DAPs and AHPs were measured from the RMP.

The term rectification was used to refer to two areas of the V-I curve (voltage as a function of current) where the curve deviates from linearity (approximately 10 mV positive and 10-20 mV negative to the RMP; Figure 4B). The result of rectification is a sag in the voltage deflection produced by a current step from the RMP to one of these two areas of membrane potential (Figure 4A). Overshoots were defined as a transient depolarization which occurs immediately after a hyperpolarizing current pulse (Figure 4A).

Antidromic action potentials were distinguished from orthodromic action potentials on the basis of latency of the action potential at

threshold. Antidromic action potentials fire at the onset of the EPSP, whereas orthodomic action potentials occur during the EPSP (Figure 16, 20).

DATA ANALYSIS

After a cell was impaled, membrane potential, action potential amplitude, and other measures of cell viability were monitored. Action potential amplitude was calculated from action potentials elicited by orthodromic stimuli, and was measured from the RMP. The input resistance (R_{in}) was defined by the voltage deflection produced at steady state in response to a 0.25 nA, 150 ms hyperpolarizing current pulse. The membrane time constant was defined as the time to reach 63% of the steady state voltage deflection produced by a 0.25 nA, 150 ms depolarizing current pulse.

The reversal potentials (E_{rev}) of GABA responses and synaptic responses were determined from a plot relating the membrane potential at which the response was elicited to the amplitude of the response at that membrane potential. Least squares linear regression analysis was used to determine the membrane potential at which the amplitude of the response would be zero, which is the reversal potential. The amplitudes of synaptic potentials were measured from the membrane potential to the peak of the responses. $GABA_s$ responses were measured from the membrane potential to the response at a fixed time (50 or 100 ms) after the onset of the pressure pulse of GABA. The $GABA_d$ responses were measured similarly, but the time point was 0.5-2 sec after the onset of the pulse of GABA. For each reversal potential determination, timepoints were chosen where the most negative (in the case of the $GABA_s$ response) and most positive (in the case of the $GABA_d$ response) reversal potentials

were calculated. This was necessary because of the contamination of each response at their peaks by the other type of response.

The method of Ginsborg (1973) was used to determine the reversal potential of the response to baclofen (Figure 22). This method entails use of the following equation:

$$V_1 - V = [r/(r + R)] (e - V)$$

where V_1 is the membrane potential during the response to baclofen, V is the RMP in control, r is the control R_{in}, R is the R_{in} during the response to baclofen, and e is the reversal potential.

DRUG APPLICATION

1. Pressure Application

GABA (10 mM in 0.9% sodium chloride, Sigma) and (-)-baclofen (1-100 uM in 0.9% sodium chloride or 1-10 mM in deionized water, Ciba-Geigy Pharmaceuticals, Summit, NJ) were applied by pressure ejection using a Picospritzer (6-38 psi, 2-500 ms, General Valve Corp.. Fairfield, NJ; Scharfman and Sarvey, 1985a, b, and d). The (-) isomer of baclofen is most potent (Bowery et al., 1985). Pressure pipettes were pulled on a vertical pipette puller (Narashige, Tokyo, Japan) using 1.5 mm O.D., 0.75 mm I.D. glass, of the same type as recording electrodes. Doses of GABA were increased by increasing pulse duration while maintaining a fixed pressure, or increasing pressure while keeping pulse duration constant.

GABA was applied up to 500 um from the intracellular electrode. Specifically, this was the distance from the point on the surface of the slice where the intracellular recording electrode was located, to the point on the surface of the slice where the pressure pipette containing GABA was located, and was measured with an ocular micrometer. The

recording electrode and pressure pipette were not perpendicular to the slice surface; in most cases they were held by micromanipulators which were angled towards each other, or in the same direction. Therefore, it is likely that the distances measured at the surface of the slice were overestimates of the actual distance between the tips of the recording electrode and pressure pipette. The tip of the recording electrode was typically 50-250 um below the slice surface. The GABA pipette was advanced through the slice in 5-50 um steps to find the depth at which the response with the fastest onset and largest conductance increase was elicited, which was usually similar to the depth of the intracellular electrode.

The GABA pipette was said to be at the soma when the tips of the GABA pipette and the intracellular electrode were less than 50 um apart on the surface of the slice. In these experiments the recording electrode was used as a marker of the soma. The recording electrode may not have been an accurate marker in some cases, since it is possible that dendritic impalements were made. However, it is unlikely that dendritic impalements were made, because in identified neurons the distance of the recording electrode from the PIA and WM measured during the experiment was the same as the distance of the soma from the PIA and WM after staining and fixation. Furthermore, the dendrites of most neurons are very small relative to somata, and therefore are more difficult to impale. Finally, the responses to GABA typical of somatic sites of ejection were always obtained when GABA was applied at the recording electrode, whereas movement of the GABA pipette away from the recording electrode in any direction resulted in a GABA response typical of dendritic GABA application.

Immediately before use of a pressure pipette containing GABA, the

amount of GABA ejected by a known pressure pulse was estimated. These measurements were made with an ocular micrometer by measuring the diameter of the drop of GABA which formed at the tip of the micropipette when the pipette was in the air (Scharfman and Sarvey, 1985a, b, and d). Based on these measurements, the amount of GABA ejected ranged from 1-100 femtomoles. To ensure the constancy of characteristics of the pressure pipette, GABA was applied every 60 or 90 seconds throughout the experiment. In addition, these measurements were repeated when the pipette was moved from one site of ejection to another, and after the experiment, to ensure that the pipette had not clogged.

2. Bath-application

In addition to pressure application, drugs or solutions with altered ionic constituents were added to the perfusate (bath-application). Duration of bath-application of drugs was corrected for the time period required for solutions to travel from the buffer flask in the waterbath to reach the chamber (6 min at 2 ml/min). Bicuculline methiodide (Sigma), pentobarbital (Sigma), clonazepam (Sigma), (-)-baclofen (Ciba-Geigy Pharmaceuticals, Summit, NJ), and tetrodotoxin (TTX; Sigma) were dissolved in buffer immediately prior to use. Diazepam was prepared from a solution (in 40% proylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol, Hoffman-LaRoche, Nutley, NJ), or its crystalline form, which was a gift from Dr. G. Mueller, Dept. Physiology, USUHS, Bethesda, MD). The high potassium solution contained 9 mM potassum chloride. When manganese was used the buffer was composed of the following (in mM): 124 sodium chloride, 3 potassium chloride, 2.4 manganese chloride, 0 calcium chloride, 1.3 magnesium sulfate, 26 sodium bicarbonate, and 10 glucose.

The low calcium, high magnesium solution had the same composition as control buffer except for the concentration of calcium, which was omitted, and magnesium sulfate, which was 10 mM.

RESULTS

BIOPHYSICAL PROPERTIES

One aim of this study was to characterize some of the fundamental biophysical properties of Layer V visual cortical neurons. A total of 148 Layer V visual cortical neurons were impaled. These cells had resting membrane potentials (RMPs) more negative than -50 mV (mean ± s.e.m., -66.6 ± 0.64 mV), overshooting orthodromic action potentials with amplitudes greater than 65 mV (86.8 ± 1.0 mV), and input resistances (R_{in}) greater than 15 megohms (36.0 ± 1.6 megohms; Table I). The membrane time constant was 14.0 ± 0.99 ms (Table I). For comparison, recordings were taken from neurons in Layers II/III, IV, and VI of visual cortex, frontal cortex, motor cortex, and area CA1 of hippocampus. The properties of these cells did not differ from those of Layer V visual cortical neurons (ANOVA; $p>0.05$, Table I).

INTRACELLULAR STAINING

To differentiate the physiological responses of pyramidal cells from those of nonpyramidal cells, twenty-four Layer V neurons were stained by intracellular injection of the fluorescent dye Lucifer yellow. Seventeen of these cells were pyramidal and the other seven were nonpyramidal (Figure 2). Each cell fulfilled all of the criteria for identification of cell type. The range of biophysical properties, responses to stimulation and current injection, and GABA responses were not different from those of unstained cells (Table I, II, and III).

There was some variation in the morphology of pyramidal cells. For example, cell bodies were slightly pear-shaped in some cases (Figure 2, 10A), whereas others were distinctly pyramidal (Figure 11A).

TABLE I

Biophysical Properties of Cortical Neurons

	n	RMP[1] (mV)	AP amplitude[2] (mV)	R_{in}[3] (megohms)	time constant[4] (ms)
Layer V	148	-66.6±0.64 (142)	86.8±1.0 (134)	36.0±1.6 (113)	14.0±0.99 (44)
Layer V Pyramidal	17	-65.3±2.1 (16)	89.1±3.2 (16)	32.9±4.5 (15)	12.2±1.3 (8)
Layer V Nonpyramidal	7	-62.1±4.3 (7)	82.4±5.3 (5)	44.3±6.1 (6)	12.9±4.0 (3)
Layer II/III	7	-67.3±3.1 (7)	79.0±1.6 (7)	18.4±2.5 (5)	—
Layer IV	6	-65.3±7.5 (6)	81.0±6.6 (5)	22.0±1.2 (3)	—
Layer VI	24	-65.9±3.4 (24)	83.8±2.7 (21)	38.3±4.8 (15)	17.7±1.6 (4)
all visual cortical cells	185	-66.9±0.61 (179)	85.9±0.93 (171)	35.4±1.4 (137)	13.8±0.66 (59)
cells from other areas of neocortex	6	-68.3±1.2 (6)	85.5±1.1 (6)	36.9±3.0 (5)	—
area CA1 hippocampus	11	-63.1±2.3 (8)	86.3±0.63 (6)	32.0±2.7 (4)	—

All values are expressed as mean ± sem, based on the n in parentheses.

[1]RMP=resting membrane potential.

[2]AP amplitude=orthodromic action potential amplitude measured from the RMP.

[3]R_{in}=input resistance, calculated from the voltage deflection at steady state produced by a 0.25 nA, 150 ms hyperpolarizing current pulse.

[4]The membrane time constant was calculated from the response to a -0.25 nA, 150 ms pulse.

Both identified (pyramidal and nonpyramidal) and unidentified visual cortical cells were included in Layer II/III, IV, V, and VI data. Pyramidal and Nonpyramidal refer only to visual cortical cells identified by Lucifer yellow injection.

Figure 2. Pyramidal and nonpyramidal cells identified by intracellular injections of Lucifer yellow.

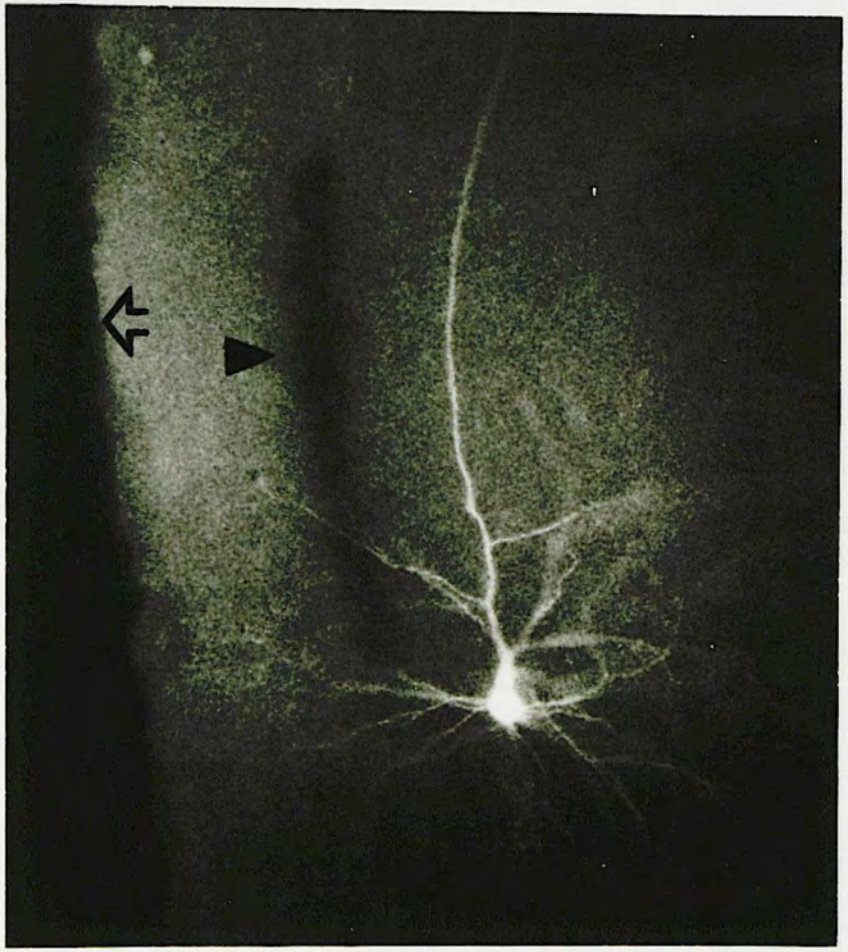
LEFT: A Layer V pyramidal cell. The apical dendrite extends towards the pia, which is directly above the photo. The edge of the slice that is normal to the pia is on the left (open arrow). The arrowhead points to a blood vessel. RMP=-75 mV, orthodromic action potential (AP) amplitude=95 mV, and R_{in}=20 megohms.

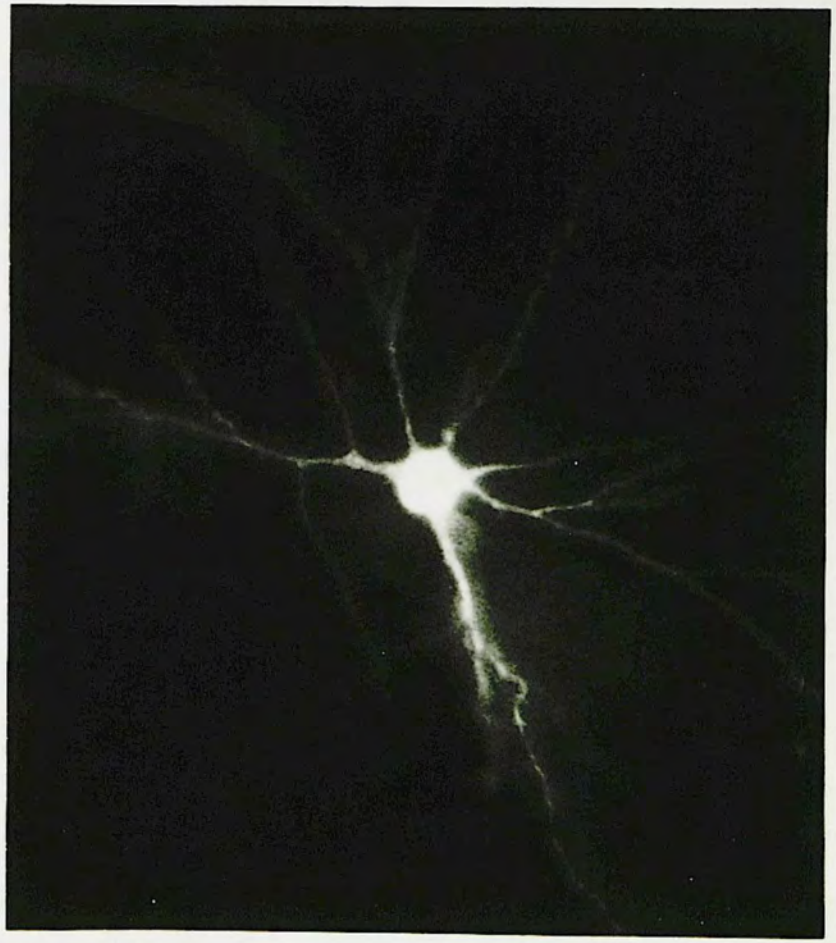
RIGHT: A Layer V nonpyramidal cell. The pial surface is directly above the photo. RMP=-83 mV, AP amplitude=98 mV,. R_{in}=-28 megohms.

Calibration bar between the photos = 100 um for the pyramidal cell on the left, and 50 um for the nonpyramidal cell on the right.

PYRAMIDAL

NONPYRAMIDAL





Dendrites branched extensively around the soma in some cells (Figure 2, 10A), whereas in others dendrites branched sparingly (Figure 11A). There were no differences in the responses of these two types of pyramidal cells to synaptic stimulation, intracellular current injection, or to GABA. In most pyramidal cells the apical dendrite ended in a tuft of branches at the pial surface, although this could not be observed in all cells. In many cells the axon could be traced to the WM. There were numerous axon collaterals that coursed perpendicular to the axon or, in two cases, towards the PIA.

The cell bodies of the nonpyramidal cells which were stained in this study were oval (Figure 10B) or circular (Figure 2, 11B). All but two of the cells were multipolar, with dendrites that radiated from all areas of the cell body, branching extensively in all directions (Figure 2, 11B). The two cells that were not multipolar were bitufted, with dendrites branching into two dendritic trees on opposite sides of the soma. One of these bitufted cells is shown in Figure 10B. Of the seven nonpyramidal cells, two were spiny (Figure 11B), one was sparsely spiny, and the others were aspiny (Figure 2, 10B). Thus, the stained nonpyramidal cells represented a variety of morphologies.

The responses of stained cells to stimulation of the WM or PIA often supported the morphological identification of these cells. For example, it is known that pyramidal cell axons, as well as afferents to these cells, course through the WM (Szentagothai, 1973; White, 1981; Jones, 1984; Peters and Kara, 1985a). As is consistent with this circuitry, it was found that morphologically identified pyramidal cells generated antidromic action potentials only following stimulation of the WM. In three cells, WM stimulation using one stimulus polarity of bipolar stimulating electrodes evoked an antidromic action potential,

whereas stimulation using the other polarity elicited an orthodromic action potential. This can be explained if one stimulus polarity preferentially activated pyramidal cell axons, whereas the other stimulus polarity activated afferent fibers to the cell.

In contrast to pyramidal cells, nonpyramidal cell axons ramify locally, within the cortex (Szentagothai, 1973; Parnavelas et al., 1977; Feldman and Peters, 1978; Peters, 1981; Jones, 1984; Peters and Kara, 1985b). This is consistent with our recordings from stained nonpyramidal cells, which show that antidromic action potentials were not elicited by stimulation of the WM. In only one case, when the PIA was stimulated, was an antidromic action potential recorded from a nonpyramidal cell. In this cell, a long, curving process, which may have been the axon, was traced from the cell body to Layer I.

Thus, use of intracellular staining was successful in separating the two broad classes of cells of the visual cortex. The characteristics of these cells were completely consistent with the descriptions made by others (Szentagothai, 1973; Parnevelas et al., 1977; Feldman and Peters, 1978; Peters, 1981; White, 1981; Jones 1984, Peters and Kara, 1985a and b).

RESPONSES TO ORTHODROMIC STIMULATION

There were three patterns of responses to stimulation, and all three could be elicited by stimulation of either the PIA or the WM (Figure 3, Table II). All three were apparent in unstained cells (Table II).

The first pattern was found in pyramidal cells as well as nonpyramidal cells (Figure 3A, Table II). Subthreshold synaptic stimulation evoked a monophasic, relatively short-lasting depolarization,

Figure 3. Three types of responses elicited by stimulation of PIA or WM. In A-C, stimulus intensity increases from response (1)-(4). IPSP= inhibitory postsynaptic potential, DAP= depolarizing afterpotential, AHP= afterhyperpolarization. Dashed lines indicate the RMP. Asterisks indicate stimulus artifact.

A. In a pyramidal cell, monophasic depolarizations were produced by low intensity stimulation of the PIA. (1-2). At threshold an action potential was triggered at the peak of the depolarization (3). Above threshold the action potential latency decreased and the DAP increased in amplitude (4). Cell location: Layer V. RMP=-62 mV.

B. In another Layer V pyramidal cell, a subthreshold stimulus produced a short-lasting depolarization followed by a hyperpolarization (1). As stimulus intensity increased both the depolarization and hyperpolarization were larger (2). At threshold (3), the action potential was followed by an AHP. Above threshold the action potential fired earlier and the AHP was larger (4). A DAP preceded a larger AHP at the suprathreshold stimulus strength. The stimulating electrode was located in the WM. RMP= -66 mV.

C. In this nonpyramidal cell, small but relatively long-lasting (note change in time base) depolarizations were produced by low intensity stimulation of the WM (1). At higher stimulus intensity depolarizations were multiphasic, variable from stimulus to stimulus, and long lasting (2). At threshold 1-2 action potentials fired at different points on the peaks of the depolarizations (3,4). Both DAPs and AHPs followed the action potentials. Cell location: Layer V. RMP=-67 mV.

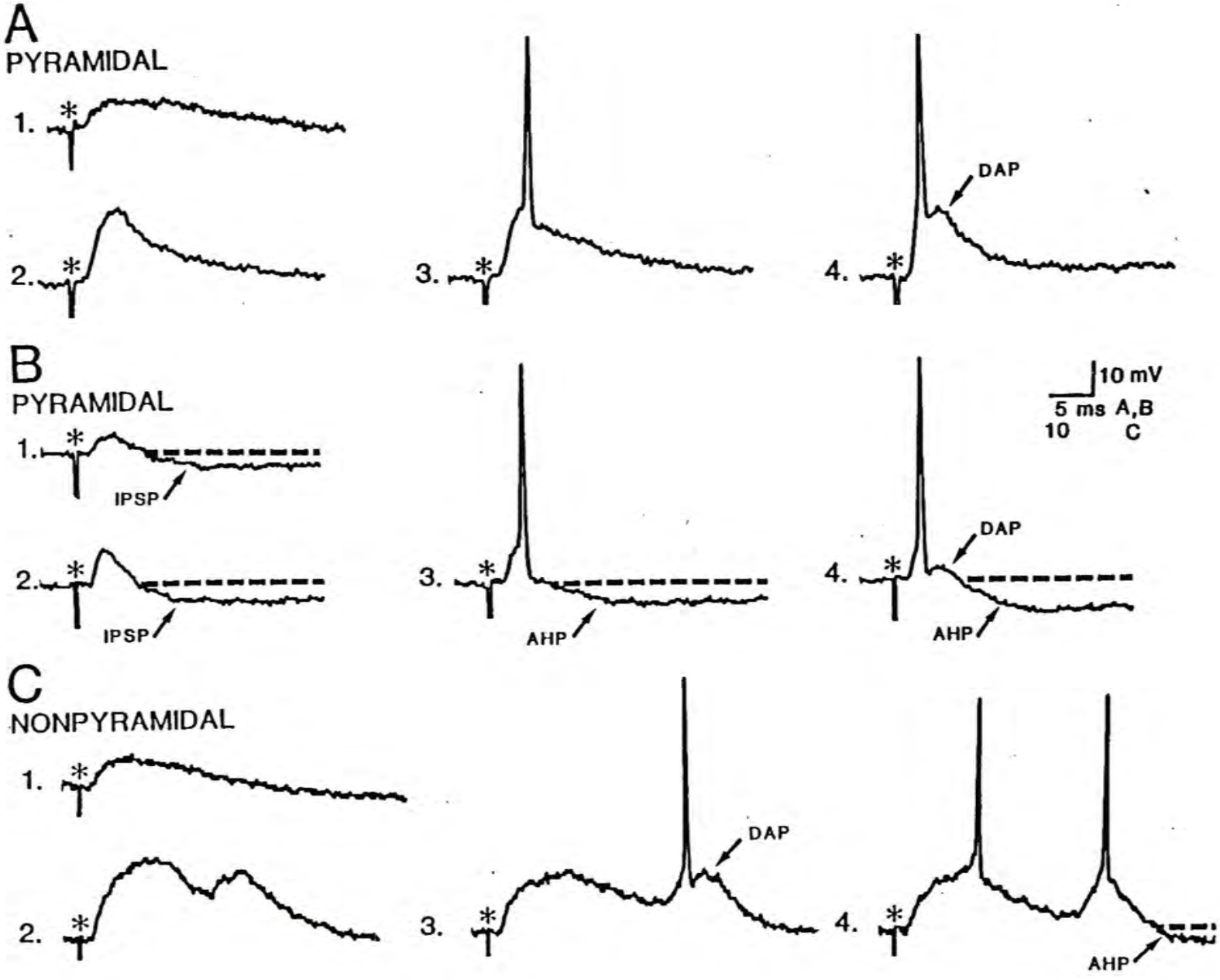

A
PYRAMIDAL
1.
2.
3.
4.
DAP
B
PYRAMIDAL
1.
IPSP
2.
IPSP
3.
AHP
4.
DAP
AHP
10 mV
5 ms A,B
10 C
C
NONPYRAMIDAL
1.
2.
3.
DAP
4.
AHP

Table II

Stimulus-response patterns in visual cortical neurons

		% of cells demonstrating the response pattern					
stimulus-response pattern:		1		2		3	
stimulus site:	n	PIA	WM	PIA	WM	PIA	WM
pyramidal cells	14	75	43	25	50	0	7
nonpyramidal cells	4	50	50	0	0	50	50
unstained	120	48	46	38	36	13	17

pattern 1:
Short-lasting depolarizations below threshold. One action potential at threshold. One or two action potentials followed by DAPs upon stimulation at twice threshold stimulus strength.

pattern 2:
Monophasic, short-lasting depolarizations followed by hyperpolarizations below threshold. One action potential at threshold. One or two action potentials followed by DAPs and/or AHPs upon stimulation at twice threshold stimulus strength.

pattern 3:
Multiphasic large, and long-lasting depolarizations just below threshold. 1-5 action potentials at threshold and at twice threshold stimulus strength. AHPs and/or DAPs.

which increased in amplitude as intensity was increased (up to 30 mV, 80 ms; Figure 3A1 and 3A2). The depolarizations appeared to be excitatory postsynaptic potentials (EPSPs), since they were graded, and at threshold an action potential was triggered at the peak of the depolarization (Figure 3A3). Furthermore, these potentials were blocked following bath-application of tetrodotoxin (1 uM, n=3). The extrapolated reversal potential for the EPSP was determined to be -28.4 $\pm$ 3.2 mV (mean $\pm$ sem, n=5). Suprathreshold (1.5-2.0 times the threshold stimulus intensity) stimuli elicited a single action potential followed by a depolarizing afterpotential (DAP; Figure 3A4).

The second pattern was observed in pyramidal cells, but not nonpyramidal cells (Figure 3B, Table II). In this case, subthreshold stimuli elicited a monophasic, short-lasting depolarization followed by a hyperpolarization (depolarizations, up to 20 mV, 10 ms; hyperpolarizations, up to 15 mV, 750 ms; Figure 3B1 and 3B2). Hyperpolarizations were sometimes biphasic (see Figure 8A, open arrows). Since action potentials were inhibited during the hyperpolarizations (see Figure 9B), it is likely that the hyperpolarizations were inhibitory postsynaptic potentials (IPSPs). The hyperpolarization had a mean reversal potential of -71.4 $\pm$ 2.4 mV (n=6). At threshold, a single action potential was followed by an afterhyperpolarization (AHP, Figure 3B). Suprathreshold stimulus intensities evoked 1-2 action potentials. In Figure 3B4, a suprathreshold stimulus produced one action potential, which was followed by a DAP as well as an AHP. In general, the AHP following suprathreshold stimulation was larger than the AHP following the threshold spike.

The third pattern of synaptic responses was found only in nonpyramidal cells (Figure 3C, Table II). When stimulus intensity was

low, a very small monophasic depolarization occurred (Figure 3C1). Following a higher intensity stimulus, a large, multiphasic, relatively long-lasting depolarization occurred (up to 40 mV, 500 ms; Figure 3C2). One characteristic of this response pattern was the variability of the response to the same stimulus. Amplitude, duration, and the number of phases of the responses to the same stimulus varied greatly. It is doubtful that this was due to an excessive stimulus frequency, since the variability persisted when the stimulus frequency was lowered to 0.05 Hz. It may be due to activation of polysynaptic pathways. Threshold stimulus strength in these cells evoked 1-5 action potentials, at a variety of latencies (Figure 3C3 and 3C4). Both DAPs and AHPs were present, and varied in amplitude and duration.

In summary, stimulation of either the PIA or WM produced three patterns of responses. Pyramidal and nonpyramidal cells shared the pattern which was typified by monophasic responses to subthreshold stimulation and one action potential at and above threshold. In contrast, the second pattern, which included subthreshold hyperpolarizations and usually one action potential, were characteristic only of pyramidal cells. The last pattern, consisting of multiphasic synaptic potentials and multiple action potentials, was unique to nonpyramidal cells.

RESPONSES TO CURRENT INJECTION

Cells were routinely tested for their responses to 150 ms hyperpolarizing or depolarizing 0.25, 0.5, 0.75, and 1.0 nA current pulses. The responses of many cells were examined in more detail, with 0.1-2.0 nA, 100-600 ms pulses. Pyramidal cells and nonpyramidal cells could not be differentiated on the basis of their responses to current pulses (Table III).

Figure 4. Typical responses to hyperpolarizing and depolarizing current pulses in three different cells.

A. (1)-(3) Responses to hyperpolarizing current pulses (-0.1, -0.45, and -0.75 nA, 150 ms) are shown. As current was increased rectification during the pulse and the overshoot (arrow) following the pulse also increased. Terminology is explained in Materials and Methods. (4) A 0.1 nA, 150 ms depolarizing current pulse delivered to the same cell also demonstrated rectification. For this and all other figures the bridge was balanced when all current pulses were given and whenever DC current was passed. Cell location: Layer V. RMP=-62 mV.

B. V-I curve from a representative visual cortical neuron. Data points were based on responses to depolarizing and hyperpolarizing (-3.7 to +0.3 nA) 150 ms current pulses. Peak measurements were made at the initial peak of the response, and steady state measurements were made during the plateau at the end of the response. The RMP was designated as 0 mV. Cell location: Layer VI. RMP=-70 mV.

C. Responses to suprathreshold depolarizing pulses.

(1)-(4): 1, 2, 4, and 7 action potentials were produced by 0.25, 0.5, 0.75, and 1.0 nA 150 ms depolarizing current pulses, respectively. Action potentials were truncated. Artifacts of current pulse onset and offset were clipped. Cell location: Layer V. RMP=-73 mV.

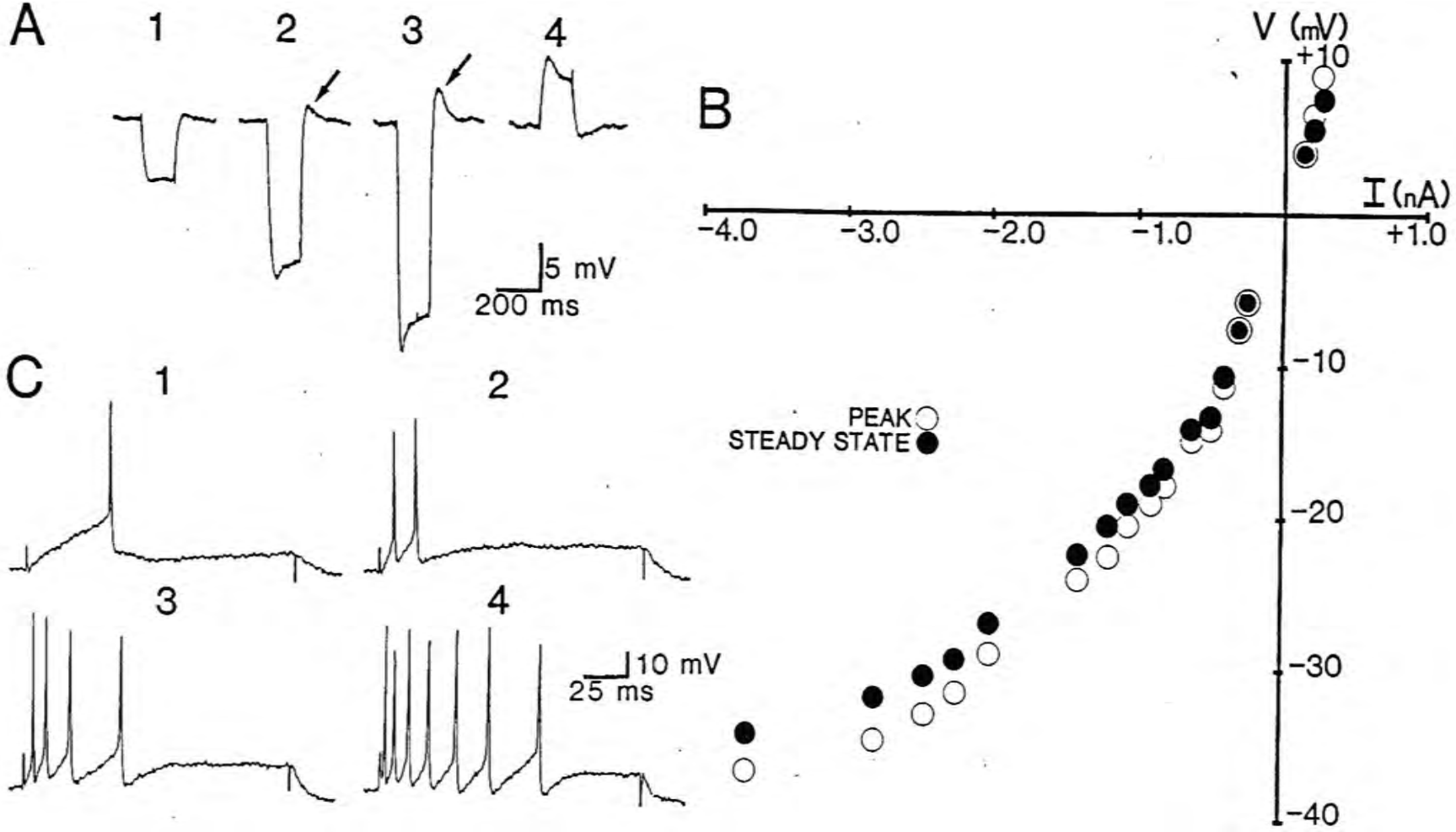

A
1
2
3
4
5 mV
200 ms
B
V (mV)
+10
I (nA)
−4.0
−3.0
−2.0
−1.0
+1.0
−10
−20
−30
−40
PEAK
STEADY STATE
C
1
2
3
4
10 mV
25 ms

Table III

Responses to Intracellular Current Injection

HYPERPOLARIZING PULSES

pulse amplitude (nA)	n	R_{in}[1] (megohms)	% of cells w/ rectification[2]	% of cells w/ overshoot[3]
0.25				
pyramidal	13	34.2±5.1	54	69
nonpyramidal	6	42.5±6.8	50	83
0.5				
pyramidal	11	22.7±2.5	82	82
nonpyramidal	6	36.2±6.8	50	100
1.0				
pyramidal	12	16.6±1.7	91	100
nonpyramidal	6	19.5±5.4	100	100

DEPOLARIZING PULSES

pulse amplitude (nA)	n	#APs[4]	AHP[5] amplitude (mV)	AHP duration (sec)
0.25				
pyramidal	13	0.84±0.22	3.2±0.84	0.68±0.16
nonpyramidal	6	0.83±0.17	3.2±0.75	0.40±0.09
0.5				
pyramidal	10	2.4±0.46	4.4±0.58	1.1±0.30
nonpyramidal	5	2.5±0.32	4.2±0.96	0.84±0.10
1.0				
pyramidal	9	4.7±1.0	5.9±0.83	1.4±0.30
nonpyramidal	2	(6.0, 6.0)	(7.5, 9.0)	(1.5, 2.0)

All pulses were 150 ms duration.
All values are expressed as mean ± sem, except the data for 1.0 nA depolarizing current pulses in nonpyramidal cells (n=2), where actual values are in parentheses.

[1] R_{in}=input resistance.

[2] Rectification during hyperpolarizing pulses: Rectification refers to a bend in the V-I curve which occurs at hyperpolarized membrane potentials. One result is that the response to a hyperpolarizing current pulse consists of an early peak followed by a decrease or "sag" in amplitude of the voltage response (see Figure 4A). A cell with rectification refers to the presence of this "sag".

[3] Overshoot: Cells with an overshoot produced a transient depolarization upon termination of the hyperpolarizing pulse.

[4] #APs=number of action potentials elicited during the depolarizing pulse.

[5] AHP= the afterhyperpolarization that occurs after a depolarizing current pulse. The amplitude of the AHP was measured from the RMP.

Responses to hyperpolarizing and subthreshold depolarizing current pulses were used to determine input resistance and generate V-I curves (membrane potential as a function of injected current). Rectification during the response to current pulses of increasing amplitude, as well as the presence of overshoots following the pulses, suggested that there were numerous voltage-dependent conductances in these cells (Figure 4A, Table III). In almost every cell, V-I curves were nonlinear (Figure 4B).

Typically, subthreshold depolarizing pulses produced anomalous rectification (Hotson et al., 1979; Figure 4A). Threshold was usually close to 0.25 nA for 150 msec duration pulses (Figure 4C). Suprathreshold pulses (0.5-1.0 nA) produced trains of up to 11 action potentials (Figure 4C; Table III). In most cells a 1.0 nA, 150 ms pulse produced 3-7 action potentials (Figure 4C). Increasing the duration of the pulse (up to 600 ms) seldom elicited more spikes, which demonstrated that these neurons had strong accommodative properties. There was no correlation between R_{in} and the number of action potentials a cell fired during the current pulse (for 1.0 nA 150 ms pulses, r=0.243, n=92 cells). Most cells produced large (up to 12 mV) and long-lasting (up to 4 sec) biphasic AHPs following suprathreshold pulses (Table III).

These data demonstrate that visual cortical neurons have many different properties which are responsible for complex responses to current pulses. These intrinsic properties are similar to many that have been described for other cortical neurons (Connors et al., 1982; Vogt and Gorman, 1982; Stafstrom et al., 1984, McCormick et al., 1985).

GABA RESPONSES

GABA was applied to 14 pyramidal cells and 6 nonpyramidal cells

of Layer V. In addition GABA was applied to 67 unstained Layer V neurons. All cells responded to GABA when it was applied within 300 um of the soma.

Two types of GABA responses appeared to exist in all cells. They could be elicited separately or in combination, and could be differentiated by their reversal potentials and by the region of the cell (soma or dendrites) where they appeared to be generated. Relatively small amounts of GABA ($\leq$1 femtomole) applied within 50 um of the soma elicited a short-lasting response which was usually hyperpolarizing ($GABA_s$ response; Figure 5A1). This response typically lasted 200-500 ms, but could last up to 3 sec. The reversal potential of the "pure" $GABA_s$ response (elicited without "contamination" by the second type of GABA response) was -71.7 $\pm$ 1.1 mV (mean $\pm$ sem, n=19). Membrane conductance increased greatly during $GABA_s$ responses, which was reflected by large decreases in resistance during the response (usually to zero, but sometimes less, as shown in Figure 5B1).

When GABA was applied to dendritic areas (50-500 um from the soma), the second type of response occurred ($GABA_d$ response; Figure 5A3). This response was always depolarizing at the RMP, and its mean reversal potential was -49.3 $\pm$ 2.4 mV (n=14). Membrane conductance increased during $GABA_d$ responses (Figure 5B3), but usually to a lesser extent than during $GABA_s$ responses. The duration of $GABA_d$ responses was usually 0.5-1 sec, although in some cases it lasted up to 6 sec. It is important to note that these responses might have produced large conductance increases, but if the response was generated in dendrites, the conductance change recorded by an intracellular electrode in the soma might be small. This could also have an effect on reversal potential calculations; therefore, -49.3 mV should only be considered an estimate

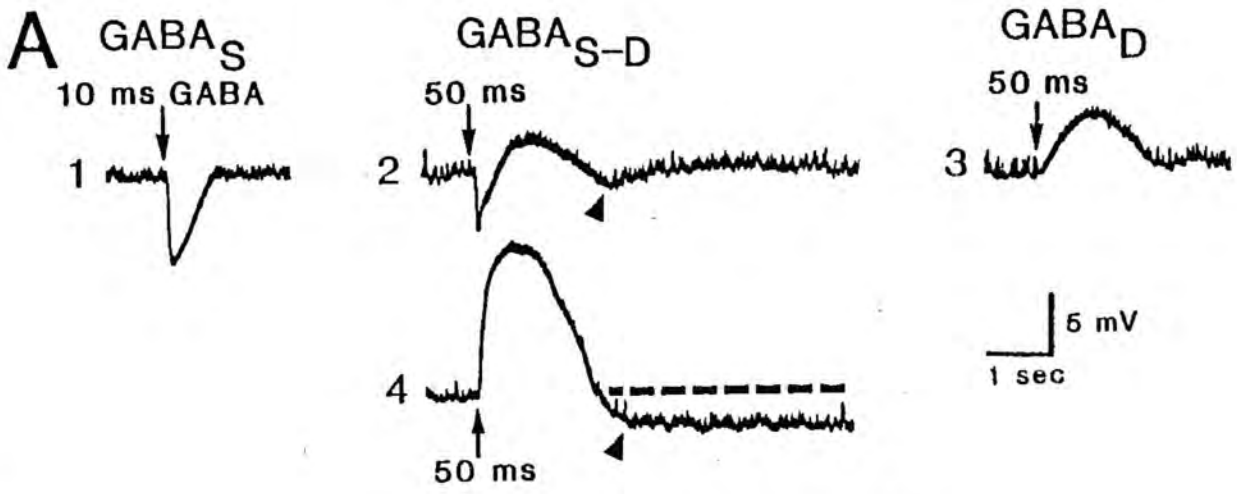
Figure 5. GABA responses of visual cortical neurons.

A. (1) $GABA_s$ response. When GABA (10 ms, 25 psi, at the arrow) was ejected less than 50 um from the soma, a hyperpolarization was produced. (2) $GABA_{s-d}$ response. When GABA (50 ms) was ejected in dendrites (150 um toward the WM from the soma) of the same cell, a hyperpolarization was followed by a depolarization. Note that a small hyperpolarization followed the depolarization (arrowhead).

(3) $GABA_d$ response. A depolarization was recorded following ejection of GABA (50 ms) at another dendritic site (100 um towards the PIA from the soma).

(4) Following ejection of GABA in (2), the cell was hyperpolarized with DC current to -70 mV (broken line). A 50 ms pulse produced a depolarization and was followed by a small, long lasting hyperpolarization (arrowhead). As was typical of this response, the late hyperpolarization was more evident at the hyperpolarized membrane potential. Cell location: Layer V. RMP=-64 mV.

B. GABA responses are accompanied by an increase in conductance. (1) In a different cell from A, GABA (10 ms, 10 psi) was ejected at the soma. (2) A 35 ms pulse of GABA was ejected 75 um from the soma towards the WM (2), and 250 um from the soma towards the PIA (3). Constant current pulses (0.2 nA for (1), 0.3 nA for (2) and (3), all 50 ms duration) were delivered at 3 Hz during the GABA responses. The decreases in amplitude of the voltage deflections in response to constant current pulses indicates a large increase in conductance during all GABA responses. Cell location: Layer V. RMP=-56 mV.


A
GABA$_S$
10 ms GABA
1
GABA$_{S-D}$
50 ms
2
4
50 ms
GABA$_D$
50 ms
3
5 mV
1 sec

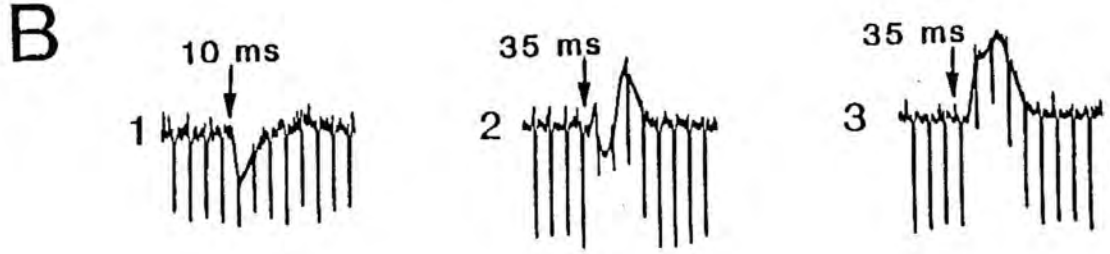
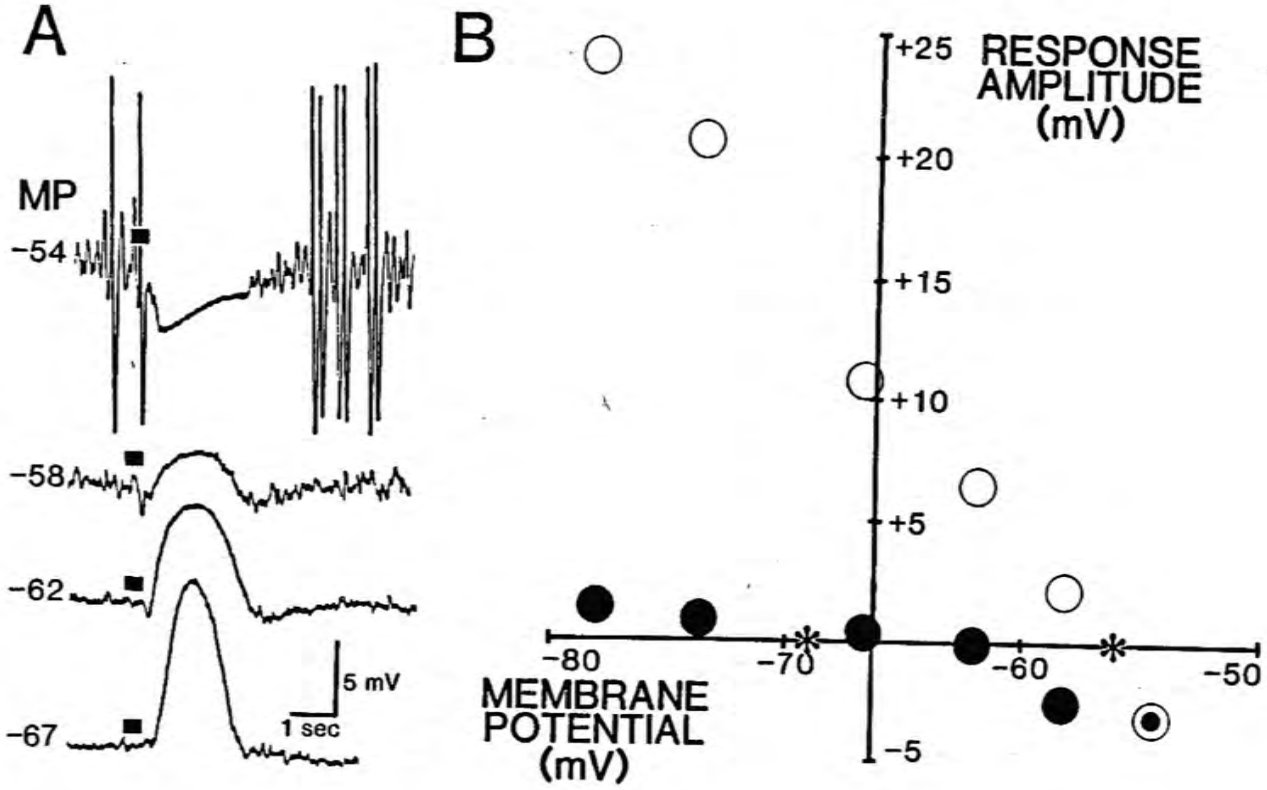

B
10 ms
1
35 ms
2
35 ms
3

Figure 6. The reversal potentials of GABA responses.

A. A $GABA_{s-d}$ response followed by a late hyperpolarization (indicated by the arrowheadrecorded at different membrane potentials. A fixed pulse of GABA (400 ms, 37 psi, solid bar) was ejected near the soma. When the membrane potential was -54 mV, GABA inhibited spontaneous action potentials (truncated by chart recorder).

B. The amplitudes of the responses were plotted against the membrane potentials at which they were elicited. A least squares regression analysis revealed that the reversal potential in this cell was -69.0 mV for the initial ($GABA_s$, filled circles) phase and -54.6 mV for the late ($GABA_d$, open circles) phase in this cell. The amplitudes of the GABA responses were measured at an early (100 ms following the onset of the GABA pulse) and a late (1.5 sec) point during the GABA response to estimate the reversal potentials of the $GABA_s$ phase and the $GABA_d$ phase. Responses were not measured at their peaks because of the apparent contamination of the two phases at these points. These time points were chosen because the most negative (in the case of the $GABA_s$ response), and most positive (in the case of the $GABA_d$ response) reversal potential occurred at this time. Cell location: Layer V. RMP=-71 mV.


A
MP
-54
-58
-62
-67
5 mV
1 sec
B
+25
+20
+15
+10
+5
-5
RESPONSE AMPLITUDE (mV)
-80
-70
-60
-50
MEMBRANE POTENTIAL (mV)

of the reversal potential of the $GABA_d$ response.

When GABA was ejected within 250 um of the soma, both types of response were usually observed in combination (Figure 5A2 and 5B2). If ejections were made close to the soma, the $GABA_s$ response preceded the $GABA_d$ response ($GABA_{s-d}$ response, Figure 5A2). In cases when GABA was applied farther from the soma, the $GABA_s$ response sometimes occurred with a delay, so that it appeared to be superimposed on the $GABA_d$ response (Figure 7B2). When $GABA_{s-d}$ responses were elicited, the reversal potential of the $GABA_s$ component was slightly less negative than the pure $GABA_s$ response (-65 to -70 mV; Figure 6), and the reversal potential of the $GABA_d$ component was slightly more negative than the pure $GABA_d$ response (-50 to -55 mV; Figure 6).

Finally, GABA produced a small, long-lasting hyperpolarization that followed a $GABA_s$ or $GABA_d$ response (Figure 5A2, 5A4, 6). This response was not evident in all cells, perhaps because the doses of GABA were usually kept small, and this response appeared to require larger amounts of GABA to be activated. In some cases it might have been masked by the $GABA_d$ response, which partially obscures the $GABA_s$ response in some cases (Figure 7B1, far right). This late hyperpolarization typically lasted 2-4 sec, but in some cases it lasted as long as 10 sec. It could be elicited by either somatic or dendritic GABA ejections and was accompanied by an increase in conductance. This response was difficult to reverse, in part because the reversal potential (extrapolated mean $\pm$ sem=-79.8 $\pm$ 1.7 mV, n=9) was located in a region where the V-I curve was nonlinear (Figure 4B), and in part because the response appeared to decrease in amplitude as the cell was depolarized by intracellular current injection (compare Figure 5A2 and 5A4). It is possible that the response is voltage-dependent.

Bath-application of tetrodotoxin (1 uM, n=3), or a solution containing either (1) 0 mM calcium chloride and 2.4 mM manganese chloride or (2) 0 mM calcium chloride and 10 mM magnesium sulphate (n=3), blocked synaptic transmission without affecting GABA responses. Therefore, it appears that GABA responses are generated postsynaptically.

Thus, there appear to be three responses to GABA which can be elicited in visual cortical neurons. The $GABA_s$ response is elicited when GABA is ejected on the soma, is associated with a large conductance increase, and has a reversal potential of approximately -70 mV. The $GABA_d$ response occurs following dendritic ejection of GABA, is also associated with a conductance increase, and reverses at approximately -50 mV. Finally, the late hyperpolarization is evoked when GABA is applied to the soma or dendrites, is accompanied by a small conductance increase, and reverses at -80 mV.

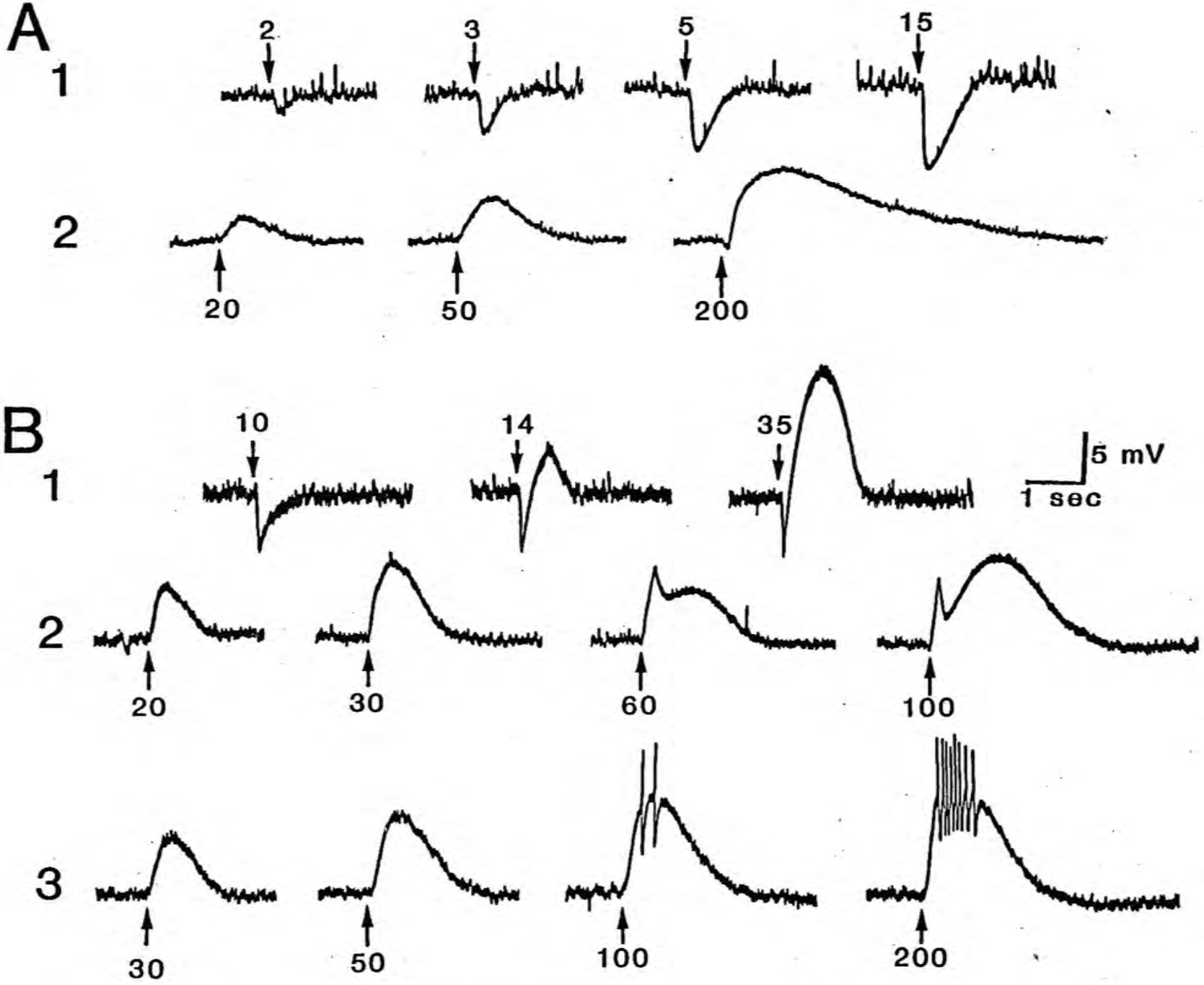
DOSE-DEPENDENCE

The membrane potential and conductance changes after GABA application were clearly dose-dependent, regardless of whether the response was hyperpolarizing, depolarizing or both (Figure 7). However, the ways in which changes in the dose affected the response were not always the same. In most cells an increase in dose produced an increase in the amplitude, duration, and conductance change of the response (Figure 7A). However, sometimes an increase in dose added a second phase to the response without a dramatic change in the first phase. This was often the case when GABA was applied to the soma, where large amounts of GABA often added a depolarizing phase to a hyperpolarizing response (Figure 7B1). When large amounts of GABA were applied in the dendrites, a hyperpolarization appeared in the middle of a depolarization (Figure

Figure 7. Dose-dependence of GABA responses.

A. Typically, an increase in duration of a pressure pulse of GABA increased the size of the GABA response. From left to right, the dose of GABA was increased by increasing the pulse duration without changing pressure. Pulse duration (ms) is indicated above the arrows, which mark when GABA was applied. 1) Dose-dependence of a $GABA_s$ response. 2) In the same cell, dose-dependence of a $GABA_d$ response. pressure= 28 psi. Cell location: Layer V. RMP=-66 mV.

B. Other dose-response relationships. 1) GABA was applied to the soma of a Layer V pyramidal cell. In this cell the pulse duration was constant (50 ms) and pressure was increased from left to right. At 10 psi a 50 ms pulse elicited a $GABA_s$ reponse. At higher pressures the 50 ms pulse produced a $GABA_{s-d}$ response. Pressure (psi) is indicated above the arrows. RMP=-59 mV. 2) GABA was ejected 100 um lateral to the soma of a different cell in Layer V. Short duration pulses elicited a $GABA_d$ response whereas longer duration pulses produced a multiphasic depolarizing response. RMP=-75 mV. 3) GABA was ejected 150 um from the soma towards the PIA. Increasing pulse duration increased the amplitude of the response up to a point, and then further increases in pulse duration did not affect the size of the response. Instead, it elicited spontaneous action potentials on the peak of the depolarization. A further increase in GABA pulse duration elicited more action potentals. This cell was a Layer V nonpyramidal cell. Action potentials were truncated by the chart recorder. RMP=-63 mV.


A
1
2
3
5
15
2
3
5
15
2
20
50
200
B
1
2
3
10
14
35
5 mV
1 sec
20
30
60
100
30
50
100
200

7B2). The dose-response relationships illustrated in Figure 7B1 and 7B2 could be explained by diffusion of excess GABA to the soma or dendrites, since mixed responses were only elicited following ejection of relatively large doses of GABA. It could also be explained if both $GABA_s$ and $GABA_d$ responses could be generated in the proximal dendrites.

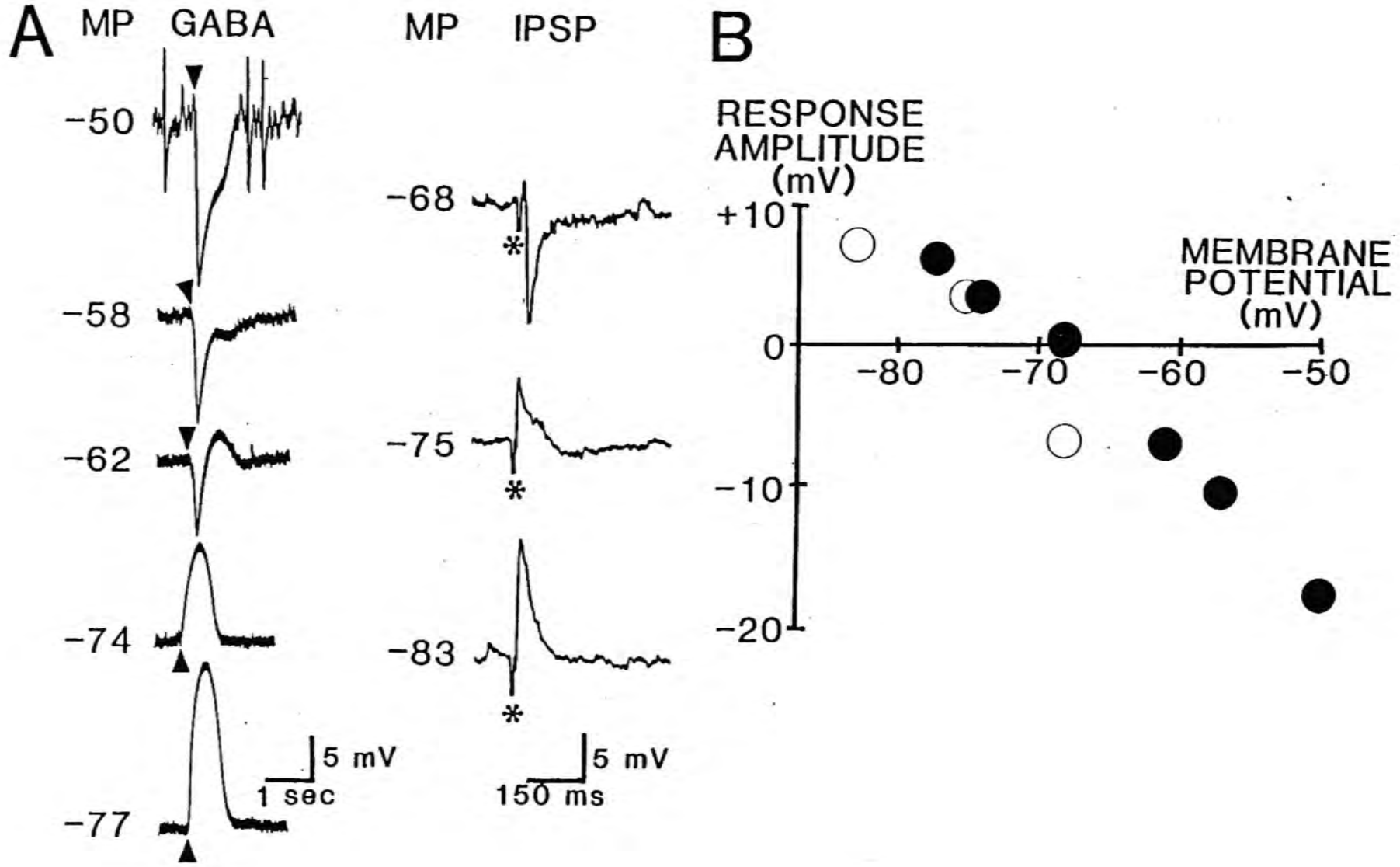
In the cell from which the data in Figure 7B3 was taken, an increase in dose of GABA induced action potential firing at the peak of a $GABA_d$ response. Depolarization of the cell to this membrane potential by passing DC current through the recording electrode induced spontaneous firing. Therefore, spontaneous firing during the $GABA_d$ response was probably due to depolarization of the cell to threshold. An indirect facilitatory effect on excitatory inputs could also have been involved. GABA could have facilitated tonic excitatory inputs to certain dendritic trees, by shunting the actions of inhibitory inputs to the dendritic tree to which it was applied.

THE $GABA_S$ RESPONSE AND THE IPSP

Synaptic responses and responses to GABA were compared to explore the possibility that GABA is a transmitter in the visual cortex. The similarities between the $GABA_s$ response and the IPSP suggested that GABA may be the transmitter of the IPSP. As shown in Figure 8, the reversal potential of the $GABA_s$ response and the IPSP were quite similar. The mean reversal potential of the $GABA_s$ response was -71.7 ± 1.1 mV (n=19), and the IPSP reversed at -71.4 ± 2.6 mV (n=6). These reversal potentials were not significantly different ($p>0.05$, t-test), but they were different from the mean reversal potentials of the $GABA_d$ responses and the late hyperpolarization ($p<0.05$, t-test).

Other data indicate that the effects of both the $GABA_s$ response

Figure 8. The reversal potentials of the $GABA_s$ response and the IPSP.
A. Left: GABA was ejected less than 50 um from the soma of a cell located in Layer V (15 ms, 30 psi, at the arrowheads). The response to GABA is shown at five different membrane potentials. RMP=-60 mV. Right: Subthreshold stimulation of the white matter (0.05 mA, 100 usec, at the asterisk) in another Layer V cell produced an IPSP, which is shown at three different membrane potentials. RMP=-70 mV. The open arrows indicate the late phases of the GABA response and the IPSP.
B. The amplitudes of the GABA response (filled circles), measured 100 ms after the onset of the pulse of GABA, and the amplitudes of the IPSP (open circles), measured 40 ms after stimulation, were plotted against membrane potential. The reversal potential of the $GABA_s$ response was -69.4 mV, and the reversal potential of the IPSP was -74.0 mV, determined by least squares regression analysis.


A
MP GABA
MP IPSP
−50
−58
−62
−74
−77
−68
−75
−83
5 mV
1 sec
5 mV
150 ms
B
RESPONSE AMPLITUDE (mV)
+10
0
−10
−20
−80
−70
−60
−50
MEMBRANE POTENTIAL (mV)

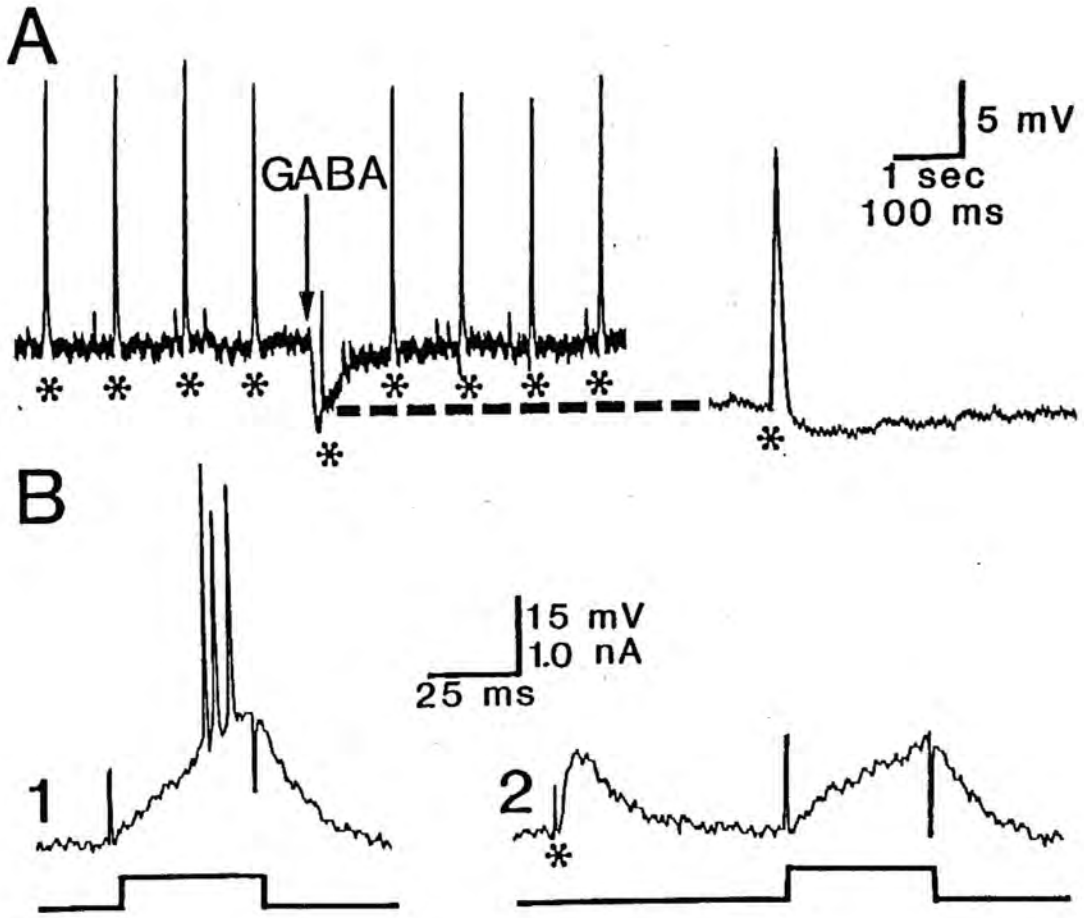
and the IPSP are inhibitory. When an orthodromic action potential was triggered at any time during a pure $GABA_s$ response the action potential was blocked (Figure 9A; n=8). Action potentials evoked by intracellular current injection (0.03-0.05 nA, 35-50 ms, n=5) were also blocked during $GABA_s$ responses. EPSPs were greatly reduced in amplitude (n=10). Inhibition was not due to a change in membrane potential, since equivalent inhibition was not invoked by shifting the membrane potential to the potential where the test stimulus was triggerred (Figure 9B). Like the $GABA_s$ response, the IPSP was inhibitory, when it was tested in an analogous manner (Figure 9B).

Although the $GABA_s$ response was inhibitory, this was not necessarily the case for the $GABA_d$ response. For example, while the $GABA_d$ component of a $GABA_{s-d}$ response was always inhibitory (see also Scharfman and Sarvey, 1985a), the pure $GABA_d$ response sometimes had no effect. In a few cases the effect of the $GABA_d$ response actually appeared to be excitatory, in that spontaneous firing occurred at the peak of the $GABA_d$ response (Figure 7B3). This seemed to be merely an effect of the membrane potential change, since when DC current was used to raise the membrane potential to the same point, these cells also fired spontaneous action potentials, and at the same frequency as when GABA depolarized the cell (data not shown).

Both the $GABA_s$ response and the IPSP were often followed by a second hyperpolarizing phase. The late phase of the $GABA_s$ response has been referred to as the late hyperpolarization. The late hyperpolarization and the late phase of the IPSP had similar characteristics. For example, their reversal potentials were similar (late hyperpolarization: -79.8 mV; late IPSP: near -80 mV in Figure 8A). These data suggest that GABA may the transmitter of both the early and

Figure 9. The $GABA_s$ response and the IPSP are inhibitory.
A. WM stimulation produced an orthodromic action potential (0.07 mA, 100 us, at the asterisks). GABA was applied less than 50 um from the soma (10 ms, 28 psi, at the arrow). During the GABA response the same stimulus produced an EPSP. Inset at right: When the cell was hyperpolarized by current injection to the membrane potential reached during the GABA response where the EPSP was elicited (broken line), the same stimulus produced an action potential, indicating that the inhibition of the action potential by GABA was not merely a result of a change in membrane potential (note faster time base). Action potentials were truncated by the chart recorder. Cell location: Layer V. RMP=-77 mV.
B. (1) In another cell, a 0.4 nA, 45 ms depolarizing current pulse (lower trace) though the recording electrode evoked three action potentials. (2) Stimulation of WM (0.45 mA, 100 usec) produced an EPSP followed by an IPSP. The IPSP is not evident because the membrane potential at which this response was evoked was -70 mV,

the reversal potential for the IPSP in this cell. During the IPSP the current pulse which produced three action potentials in (1) did not evoke any action potentials. Action potentials ere truncated. Cell location: Layer V. RMP=-70 mV.


A
GABA
5 mV
1 sec
100 ms
B
15 mV
1.0 nA
25 ms
1
2

late phases of the IPSP.

SIMILARITY OF GABA RESPONSES ELICITED FROM CELLS OF DIFFERENT MORPHOLOGY AND LOCATION

A central question of this study was whether cells of different morphology differed in their responses to GABA. This was found not to be the case. Responses to GABA of morphologically identified pyramidal and nonpyramidal cells were quite similar (Figure 10 and 11). In addition, cells located in different layers (Figure 12A), and in different areas of the cortex (Figure 12B), had a similar pattern of GABA responsiveness. In Figure 10A, GABA responses were recorded from a neuron which was later identified as a Layer V pyramidal cell. When GABA was ejected 75 um towards the PIA from the soma, a $GABA_{s-d}$ response was produced, followed by a long-lasting hyperpolarization. In Figure 10B, a very similar response to GABA was recorded from another Layer V cell in a different slice. In this case intracellular injection of Lucifer yellow revealed that the cell was nonpyramidal. However, despite the different morphology, there was a very similar response to GABA.

In Figure 11, similar GABA responses are shown from two other cells, which were morphologically identified as pyramidal (Figure 11A) and nonpyramidal (Figure 11B). As was shown in Figure 10, the responses to GABA were quite similar, despite the morphological differences between cells. In both cells, a $GABA_d$ response was elicited when GABA was ejected in dendrites (site 1). When a relatively small dose of GABA was ejected very close to the soma (site 2) a pure $GABA_s$ response was elicited. A large dose of GABA at the same site (Figure 11A) or 50 um away (Figure 11B) resulted in a $GABA_{s-d}$ response. The data in Figures 10 and 11 show that all responses to GABA can be elicited in both pyramidal

Figure 10. GABA responses of pyramidal cells are similar to those of nonpyramidal cells.

A. LEFT: A Layer V pyramidal cell. The pial surface is toward the upper right. RMP=-73 mV. Orthodromic action potential amplitude=95 mV. R_{in}= 32 megohms. Calibration for A and B = 20 um.

CENTER: A tracing of the cell on the left. GABA was applied 50 um towards the PIA (indicated by the asterisk).

RIGHT: A 25 ms pulse of GABA (15 psi, at the arrow) produced a $GABA_{s-d}$ response, followed by a long-lasting hyperpolarization.

B. LEFT: A Layer V nonpyramidal cell. The pial surface is towards the upper right. RMP=-67 mV. Orthodromic action potential amplitude=82 mV. R_{in}=34 megohms.

CENTER: GABA was applied 200 um towards the PIA.

RIGHT: A 100 ms pulse of GABA (33 psi) produced a response similar to that in A.

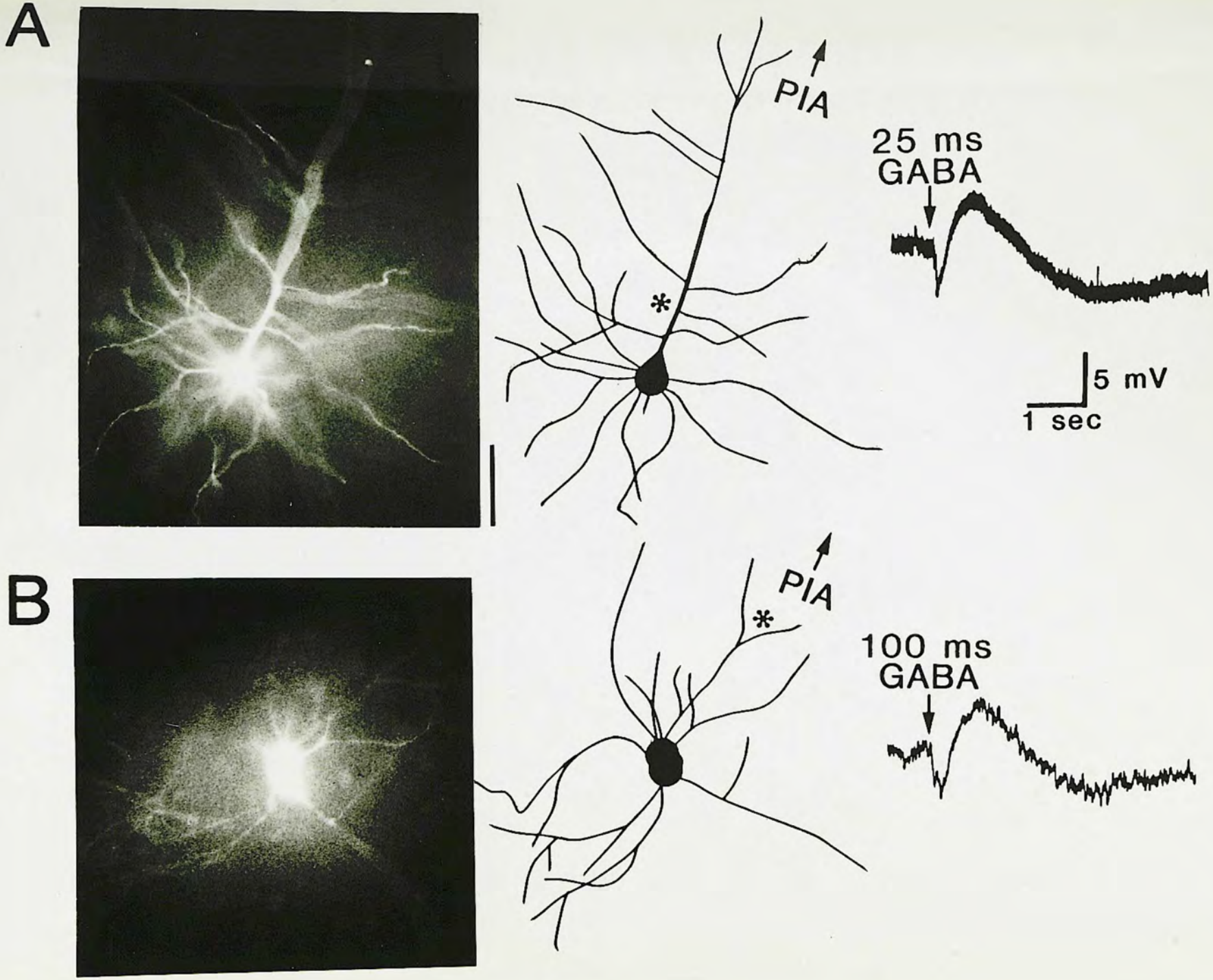

A
PIA
25 ms
GABA
5 mV
1 sec
B
PIA
100 ms
GABA

Figure 11. $GABA_s$ and $GABA_d$ responses of pyramidal cells were elicited in similar cell regions as GABA responses of nonpyramidal cells.

A. GABA responses of a pyramidal cell.

LEFT: A Layer V pyramidal cell. The pial surface is toward the upper left. RMP=-60 mV. Orthodromic action potential amplitude=84 mV. R_{in}=34 megohms. Calibration in A and B = 50 um.

CENTER: The cell on the left was traced, and the two sites of GABA application are illustrated.

Site 1: The pressure pipette was placed on the surface of the slice 100 um from the soma towards the pial surface.

Site 2: Less than 50 um from the soma.

RIGHT: Responses elicited by ejection of GABA at site 1 and site 2.

Site 1: A 50 ms duration pulse (35 psi; at the arrow) elicited a depolarization.

Site 2, left: A 30 ms pulse produced a hyperpolarization.

Site 2, right: A slightly longer pulse (50 ms) produced a $GABA_{s-d}$ response followed by a small, late hyperpolarization.

B. GABA responses of a nonpyramidal cell.

LEFT: A Layer V nonpyramidal cell. The pial surface is directly above. RMP=-60 mV. Orthodromic action potential amplitude=70 mV. R_{in}=52 megohms.

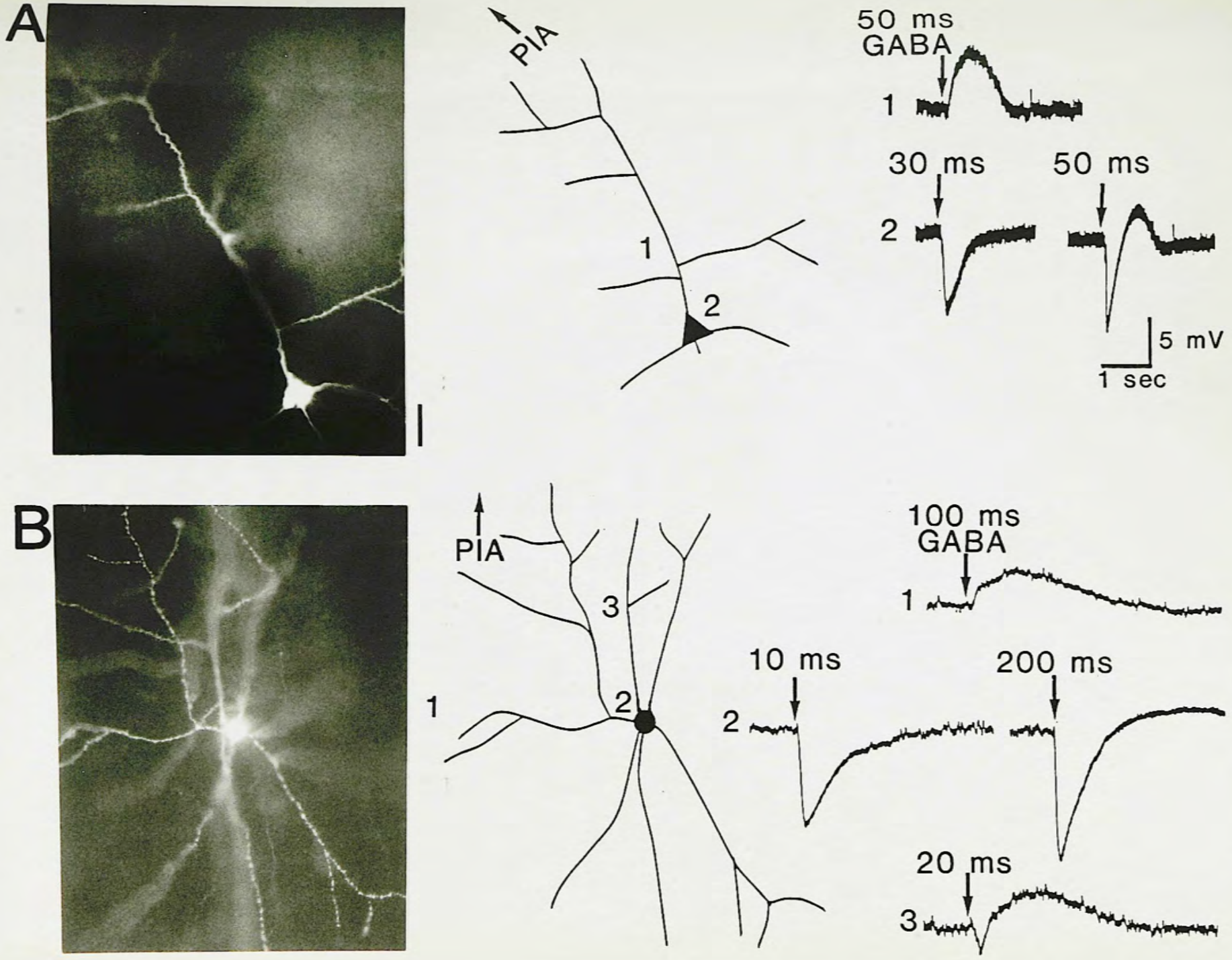
CENTER: Sites of GABA ejection are illustrated.

Site 1: 300 um lateral to the soma.

Site 2: less than 50 um from the soma.

Site 3: 150 um towards the pial surface from the soma.

RIGHT: GABA responses elicited by GABA ejection at sites 1-3. Site 2, left: 50 um deep. Site 2, right: at the surface of the slice. Pulse duration (ms) is indicated above the arrows; pressure=35 psi.


A
PIA
1
2
50 ms
GABA
1
30 ms
50 ms
2
5 mV
1 sec
B
PIA
1
2
3
100 ms
GABA
1
10 ms
200 ms
2
20 ms
3

and nonpyramidal cells.

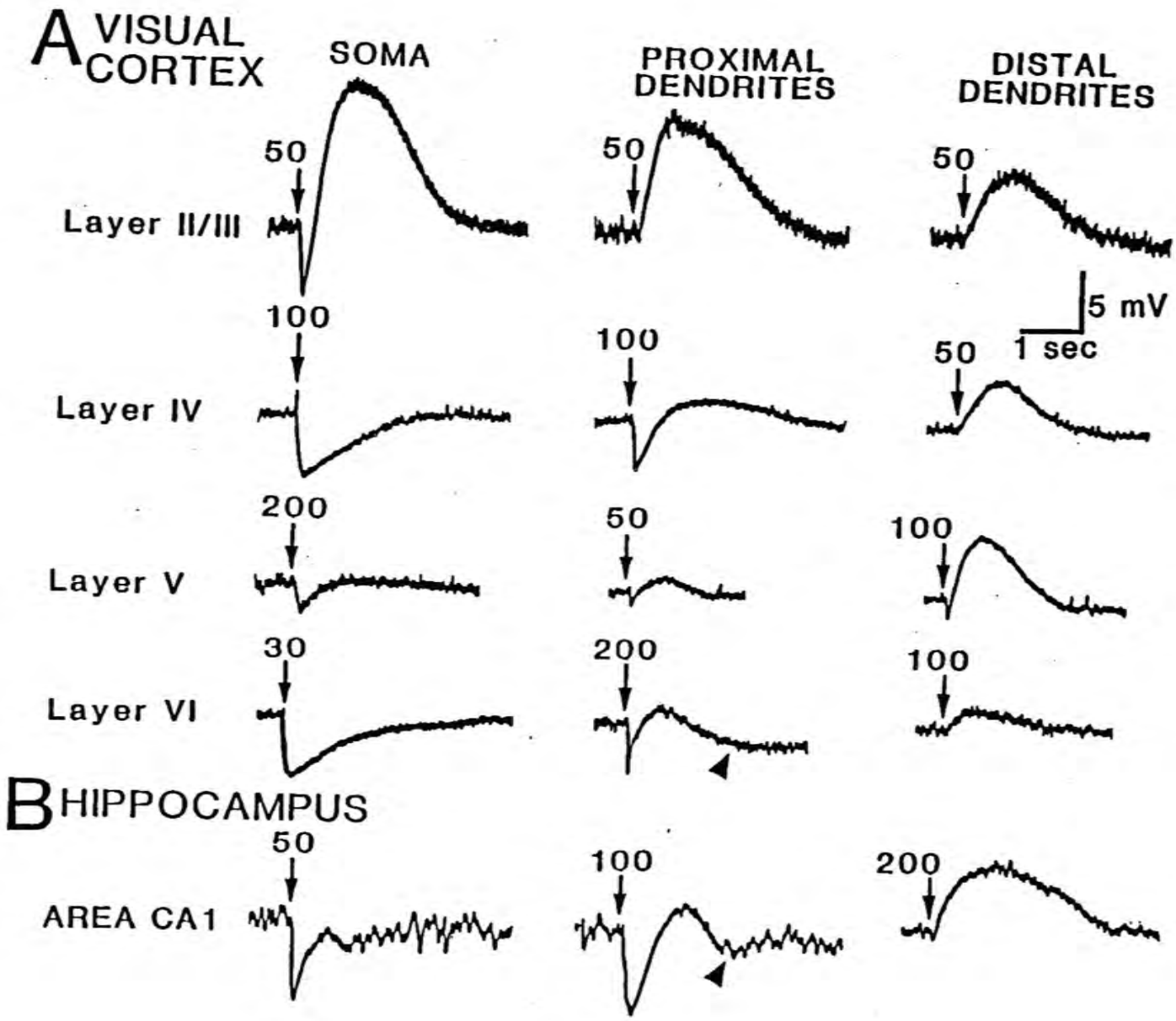
Figure 12A shows GABA responses from four different cells whose cell bodies were located in different layers of the visual cortex. In each case GABA was ejected at the soma, in proximal dendrites (0-200 um from the soma), and in distal dendrites (over 200 um from the soma). Typically $GABA_s$ responses occurred when the GABA pipette was at the soma, $GABA_{s-d}$ responses occurred when the pipette was in proximal dendrites, and $GABA_d$ responses occurred when the pipette was placed in distal dendrites. Thus, regardless of the location of the cell, the responses to GABA were similar.

To determine whether other cortical areas contained cells which responded to GABA in a similar way, cells were impaled in frontal (n=2) and motor areas (n=4), as well as area CA1 of the hippocampus, which is archicortex (n=11). The GABA responses elicited in these areas were remarkably similar to those of the visual cortical cells described above. In Figure 12B the GABA responses of a hippocampal pyramidal cell from area CA1 are shown. When the GABA pipette was placed at the soma, a $GABA_s$ response was recorded. When GABA was ejected in proximal dendrites (125 um from the soma in the apical dendritic tree, in stratum radiatum; Figure 12B) a $GABA_{s-d}$ response occurred. A $GABA_d$ response was recorded when the pipette was placed further from the soma (200 um from the soma in the basal dendritic tree, in stratum oriens; Figure 12B). The late hyperpolarization was evident after the $GABA_{s-d}$ response of the hippocampal pyramidal cell in Figure 12B (marked by an arrowhead). Note the similarity to the late hyperpolarizations (also marked by arrowheads) following the $GABA_{s-d}$ response of the Layer VI visual cortical cell in Figure 12A, and the Layer V cell in Figure 5A2.

Figure 12. GABA responses of cells in different cortical layers and areas of the cortex were similar.

A. GABA responses recorded from neurons with cell bodies located in different layers are shown. The traces in the left column are $GABA_s$ or $GABA_{s-d}$ responses elicited when GABA was applied less than 50 um from the cell soma. In the center column $GABA_{s-d}$ or $GABA_d$ responses from the same cells are shown following GABA application 50-200 um from the soma. $GABA_{s-d}$ or $GABA_d$ responses in the right column were recorded fom the same cells when GABA was ejected over 200 um from the soma. GABA was applied at the arrows. Pulse durations (ms) are indicated above the arrows. Arrowheads point to the small, late hyperpolarization which often follows $GABA_s$ or $GABA_d$ responses. RMPs for these cells were: (Layer II/III)-62 mV, (Layer IV)-55 mV, (Layer V)-66 mV, (Layer VI)-67 mV.

B. GABA responses recorded from a CA1 hippocampal pyramidal cell. GABA was applied less than 50 um from the soma (left), 100 um from the soma in stratum radiatum, which is the layer of apical dendrites (center), and 200 um from the soma in stratum oriens, which is the layer of basal dendrites (right). The GABA responses recorded from hippocampus were elicited at -60 mV to make the similarity to the $GABA_s$ and $GABA_d$ responses of visual cortical neurons evident. RMP=-70 mV.


A VISUAL CORTEX
SOMA
PROXIMAL DENDRITES
DISTAL DENDRITES
50
Layer II/III
50
50
5 mV
1 sec
100
Layer IV
100
50
200
Layer V
50
100
30
Layer VI
200
100
B HIPPOCAMPUS
50
AREA CA1
100
200

SEGREGATION OF $GABA_S$ AND $GABA_D$ RESPONSES

The data illustrated in Figures 5, 11, and 12 suggest that application of GABA at the soma elicits $GABA_s$ responses, and application of GABA on distal dendrites evokes $GABA_d$ responses. Thus, the two processes underlying the $GABA_s$ and $GABA_d$ responses appeared to be segregated to two different areas of the cell. However, the degree of segregation was unclear, because, when GABA was applied to proximal dendrites, mixed responses occurred.

Two hypotheses could explain the data. First, both responses ($GABA_s$ and $GABA_d$) may be generated in the proximal dendrites. Second, the different processes underlying the $GABA_s$ and $GABA_d$ responses may be segregated to the soma and dendrites, respectively. The second hypothesis would predict that the mixed responses merely represent diffusion of GABA to the soma, where the $GABA_s$ component was elicited, and diffusion of GABA to the dendrites, where the $GABA_d$ component was generated.

Using depth profiles, an attempt was made to discriminate between the two possibilities. Figure 13A presents data that support the first hypothesis. The GABA pipette was 75 um from the soma at the surface of the slice. At a depth of 40 um a $GABA_s$ response was recorded, but 10 um deeper a $GABA_d$ response was elicited, and between the two depths a $GABA_{s-d}$ response was elicited, all following the same pressure pulse of GABA. The extremely close proximity of the pure $GABA_s$ and $GABA_d$ responses supports the first hypothesis, that both responses can be elicited in the same area of the cell. However, this assumes that the GABA pipette was moving along a contiguous segment of membrane, whereas it could have been passing between the soma and a dendrite branching close to the soma. Therefore, although suggestive, this is not

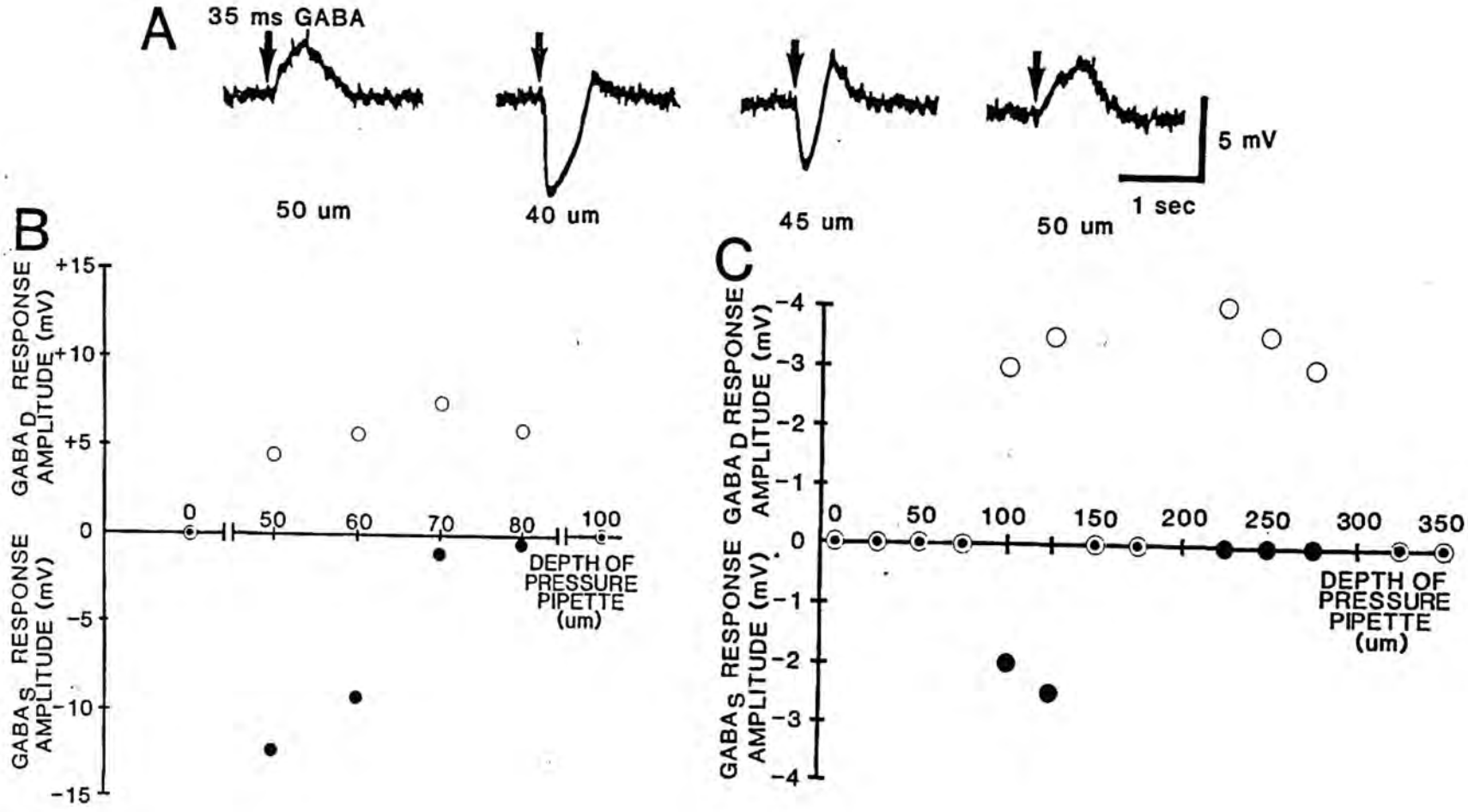
Figure 13. Depth profiles of responses to GABA ejected in the proximal dendrites.

A. Responses to a fixed pulse of GABA (35 ms, 28 psi, at the arrow) are shown after ejections at different depths in the tissue slice.

(1) At the surface, the GABA pipette was 75 um towards the WM from the intracellular electrode. The pipette was advanced to a depth of 50 um, and the 35 ms pulse of GABA produced a $GABA_d$ response. (2) The pipette was moved backwards 10 um, bringing it to a depth of 40 um, and a $GABA_s$ response occurred following the same pulse of GABA. (3) The GABA pipette was advanced 5 um, so that its depth was 45 um, and a $GABA_{s-d}$ responses was elicited. (4) The pipette was advanced 5 um deeper, bringing the GABA pipette back to its original depth of 50 um. At this point the pulse of GABA produced a similar $GABA_d$ response as was evoked in (1). The depth of the intracellular electrode was 75 um. All GABA responses were recorded at a membrane potential of -66 mV. Cell Location: Layer V. RMP=-72 mV.

B. A depth profile is plotted for data from the same cell as in A, when the GABA pipette was in another location in the proximal dendrites. Amplitudes of the $GABA_s$ (filled circles) and $GABA_d$ (open circles) responses are plotted as a function of depth of the GABA pressure pipette in the slice. At the surface of the slice the GABA pipette was 100 um towards the WM from the intracellular electrode. All GABA ejections were 50 ms duration. The membrane potential was -57 mV for all recordings.

C. In another cell, a complete depth profile was made through the entire slice. The GABA pipette was 100 um from the intracellular electrode at the surface of the slice. The depth of the recording electrode was 100 um. Near this depth a $GABA_{s-d}$ response was elicited (200 ms, 40 psi). There was no response to the 200 ms pulse when the pressure pipette was at depths of 150 and 175 um. At 225-275 um, a pure $GABA_d$ response was elicited. The membrane potential during these recordings was -58 mV. Cell location: Layer V. RMP=-70 mV.


A
35 ms GABA
50 um
40 um
45 um
50 um
5 mV
1 sec
B
GABA D RESPONSE AMPLITUDE (mV)
GABA S RESPONSE AMPLITUDE (mV)
+15
+10
+5
0
-5
-10
-15
0
50
60
70
80
100
DEPTH OF PRESSURE PIPETTE (um)
C
GABA D RESPONSE AMPLITUDE (mV)
GABA S RESPONSE AMPLITUDE (mV)
-4
-3
-2
-1
0
-1
-2
-3
-4
0
50
100
150
200
250
300
350
DEPTH OF PRESSURE PIPETTE (um)

conclusive evidence that the two responses can be elicited from the same area of the cell.

The first hypothesis was supported by other findings as well. First, some responses with a $GABA_s$ component were elicited far from the soma (Figure 10A, 11B, 12A). Second, it was often difficult to obtain a pure $GABA_s$ response at the soma. Often, when the tip of the GABA pipette was less than 50 um from the soma at the surface of the slice, a $GABA_d$ or $GABA_{s-d}$ response was elicited. Only when advancing deeper into the slice, towards the tip of the recording electrode, and by reducing the amount of GABA ejected, could a pure $GABA_s$ response be recorded (Figure 11B, site 2).

Other depth profiles supported the second hypothesis, that $GABA_s$ and $GABA_d$ responses are spatially segregated. In Figure 13B another depth profile is plotted from GABA responses of the same cell as in 13A. The GABA pipette was in a different location in the proximal dendrites. As the depth of the GABA pipette approached the level of the intracellular electrode (75 um), the $GABA_s$ response was largest. Note that the $GABA_s$ response was elicited in a much smaller area than the $GABA_d$ response, and that this area was near the depth of the intracellular electrode. (Although the depth of the GABA pipette was 50 um, not 75, when the maximal $GABA_s$ responses was elicited, it is likely that the GABA pipette was close to the soma, since the recording electrode and GABA pipette were angled at each other, and therefore were probably closer than the measurements suggest.) The fact that $GABA_s$ responses were elicited when the GABA pipette was at a depth close to that of the intracellular electrode would be expected if $GABA_s$ responses reflected responses to GABA generated at the soma.

Another example is shown in Figure 13C, which presents data from

another cell. At the depth of the recording electrode, a $GABA_{s-d}$ response was elicited following a 200 ms pulse of GABA. With a lower dose of GABA (50 ms) only a $GABA_s$ response could be recorded at this depth (data not shown). As the pressure pipette was advanced, moving away from the soma, there was no response to GABA. However, when the pipette was very deep (over 100 um from the soma), a pure $GABA_d$ response was elicited; this probably represented activation of distal dendritic GABA receptors on the same cell.

In summary, there is data to support both hypotheses, and the question of spatial segregation of the $GABA_s$ and $GABA_d$ responses is still open.

PHARMACOLOGY

The pharmacological profile of the GABA responses of visual cortical neurons was tested, using four compounds known to act at different sites on $GABA_A$ and $GABA_B$ receptors: 1) the barbiturate, pentobarbital, 2) the benzodiazepine, diazepam, 3) the convulsant, bicuculline, and 4) the antispastic drug, baclofen.

1. PENTOBARBITAL

The effects of pentobarbital on GABA responses were tested in six cells from Layer V. Pentobarbital was bath-applied (500 uM- 1 mM) while responses to pressure-applied GABA were monitored. There were no differences in the effects produced by the two concentrations.

In all cells, the amplitude, duration, and conductance increase of the $GABA_d$ responses were enhanced during bath-application of pentobarbital (Figure 14A). The reversal potential became more positive

Figure 14. Pentobarbital increases the amplitude and conductance change during $GABA_d$ responses, in both pyramidal and nonpyramidal cells.

A. CONTROL: A 50 ms pulse of GABA (7 psi, at the arrow), evoked a $GABA_d$ response. During the GABA response there was an increase in membrane conductance, as shown by a decrease in the amplitude of the response to 0.25 nA, 50 ms hyperpolarizing current pulses.

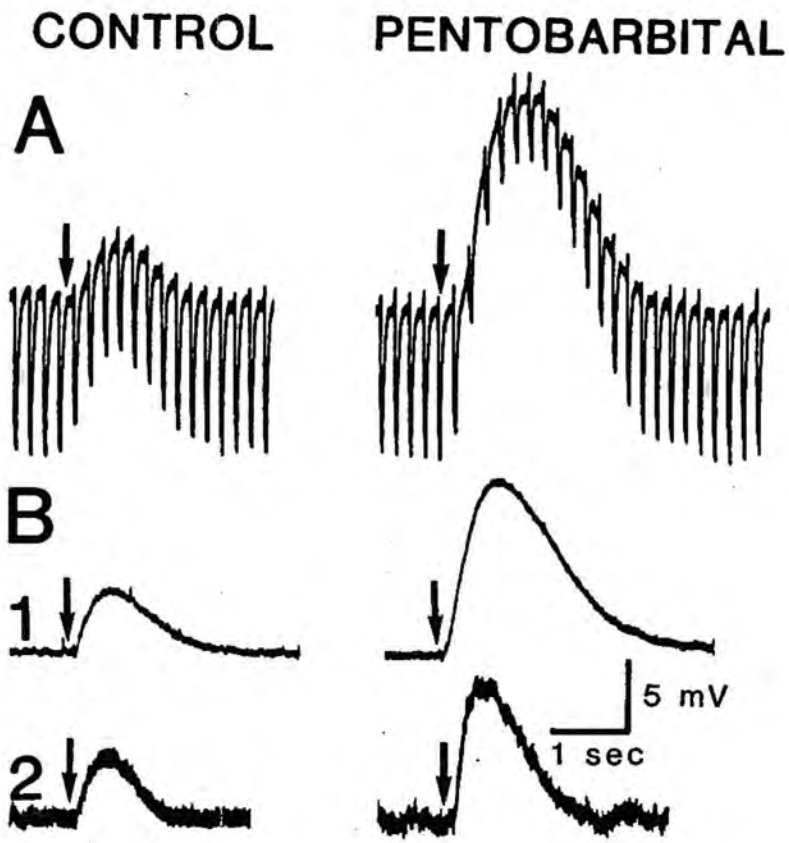
PENTOBARBITAL: After bath-application of 1 mM pentobarbital for 10 minutes, the amplitude of the GABA response and the conductance change increased. The conductance increase is actually greater than what is shown, since in this cell R_{in} increased greatly at the membrane potential reached during the depolarization elicited by GABA. Cell Location: Layer V. RMP=-70 mV.

B. CONTROL: A 15 ms pulse of GABA (36 psi) ejected 175 um towards the PIA from the soma produced a $GABA_d$ response of a Layer V pyramidal cell. A photo of the cell is shown in Figure 10A.

PENTOBARBITAL: After pentobarbital (1 mM) was bath-applied for 10 minutes the GABA response increased in amplitude. RMP=-62 mV.

C. CONTROL: A $GABA_d$ response was elicited by ejection of GABA 150 um lateral to a Layer V nonpyramidal cell (50 ms, 7 psi). This cell is shown in Figure 2.

PENTOBARBITAL: Bath-application of 1 mM pentobarbital for 12 minutes enhanced the response to GABA. RMP=-83 mV.


CONTROL
PENTOBARBITAL
A
B
1
2
5 mV
1 sec

in the four cells where it was calculated. Two of these cells were also stained with Lucifer yellow; one was pyramidal (Figure 14B) and the other was nonpyramidal (Figure 14C). The fact that their responses to GABA were affected similarly suggests that not only do pyramidal and nonpyramidal cells respond similarly to GABA, but that they also are affected similarly by GABAergic drugs.

In four cells, a $GABA_{s-d}$ response was followed by a late hyperpolarization in control. In each case, when pentobarbital was bath-applied the $GABA_d$ response became very large, but the amplitude of the $GABA_s$ component and the late hyperpolarization were decreased or blocked (Figure 15). It is difficult to determine whether the $GABA_s$ and late hyperpolarizing responses were inhibited directly by pentobarbital, or were merely obscured due to the increase in amplitude and duration of the $GABA_d$ response.

In two of the six cells, the RMP hyperpolarized 2-3 mV. The only other aspects of cell physiology that were affected by pentobarbital were the responses to orthodromic stimulation. This was examined in two cells from Layer V, and in both cells the threshold for action potential generation increased and the subthreshold response (EPSP) decreased in amplitude (Figure 16). The decrease in EPSP amplitude could not be accounted for by a decrease in R_{in} since the responses to hyperpolarizing and depolarizing pulses did not change (Figure 16C). In one of these cells, orthodromic action potentials could no longer be elicited following pentobarbital treatment (Figure 16B). In only one cell was it possible to monitor reversal of these changes during a long period of wash. In that cell all effects on GABA responses and responses to orthodomic stimulation completely reversed.

Thus, the predominant effect of pentobarbital was a dramatic

Figure 15. Decrease in amplitude of $GABA_s$ responses and the late hyperpolarization during enhancement of $GABA_d$ responses by pentobarbital. GABA was ejected less than 50 um from the soma (30 psi, at the arrow). Pulse duration was increased from left to right. Cell location: Layer V. All responses were elicited at -63 mV. RMP in control=-63 mV; -65 mV during bath-application of 500 uM pentobarbital.

CONTROL: GABA responses in control.

PB + 10′: Responses to the same ejections of GABA approximately 10 minutes after the onset of bath-application of pentobarbital (500 uM). The responses to pulses of different duration were recorded as follows: 50 ms, 9 min; 100 ms, 8 min; 170 ms, 7 min.

PB + 20′: Responses to GABA approximately 20 minutes after the onset of bath-application of pentobarbital. Responses were recorded at the following times: 50 ms, 24 min; 100 ms 23 min; 170 ms, 17 min.

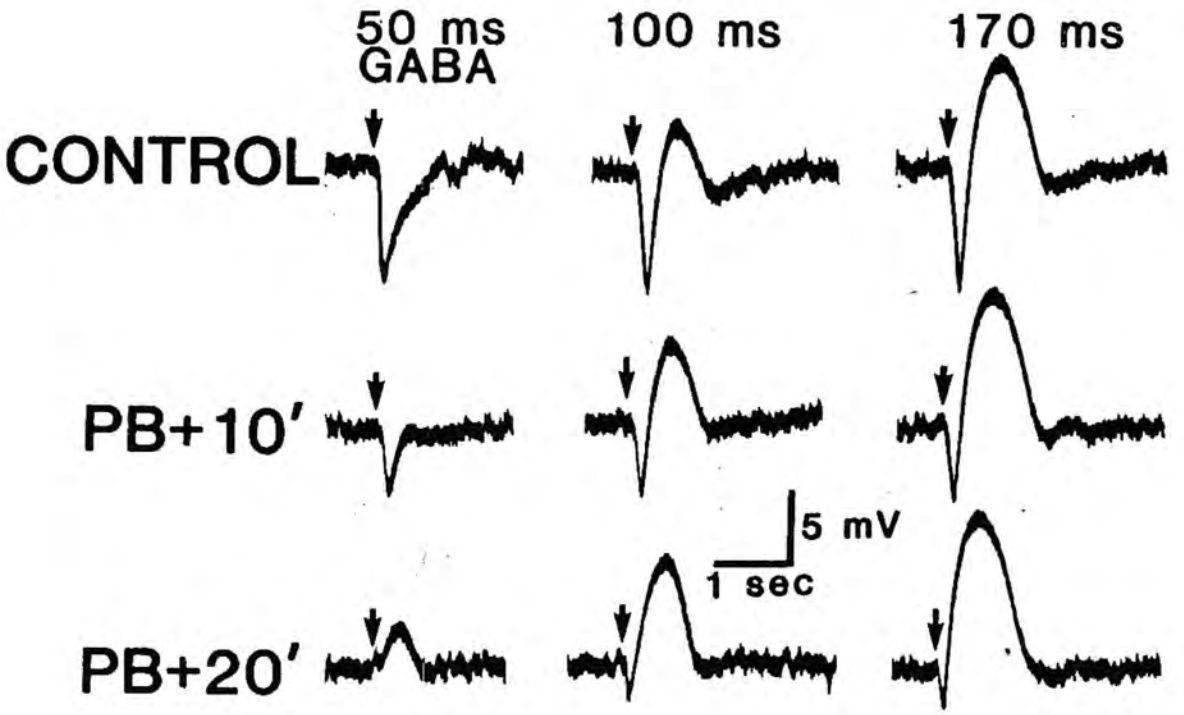
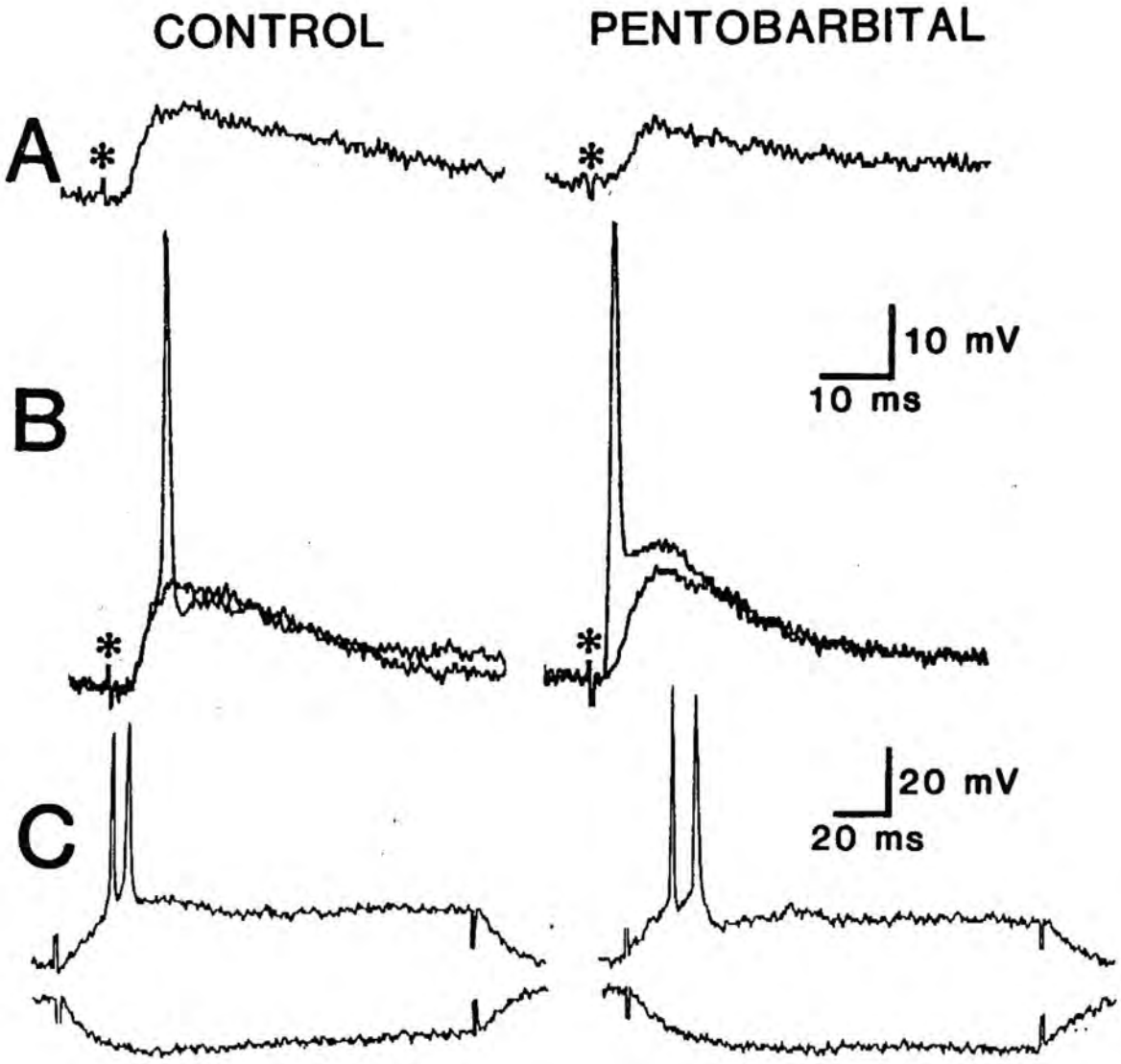

50 ms
GABA
100 ms
170 ms
CONTROL
PB+10′
PB+20′
5 mV
1 sec

Figure 16. Pentobarbital inhibits responses to orthodromic stimulation but not responses to current pulses. All responses are from the same cell. Cell location: Layer V. RMP=-70 mV. Asterisk indicates stimulus artifact. Action potentials were truncated by digitization of the trace.
A. CONTROL: An EPSP was elicited following WM stimulation (0.05 mA, 100 us). PENTOBARBITAL: Seven minutes after the onset of pentobarbital bath-application (1 mM), the EPSP amplitude had decreased.
B. CONTROL: Stimulation at threshold (0.066 mA, 100 us) elicited an orthodromic action potential or EPSP.
PENTOBARBITAL: To reach threshold, it was necessary to increase stimulus strength to 0.09 mA, 100 us. Only antidromic action potentials could be elicited.
C. CONTROL: The responses to 0.5 nA, 150 ms hyperpolarizing and depolarizing current pulses are shown before bath-application of pentobarbital.
PENTOBARBITAL: The responses to the same current pulses did not change during bath-application of pentobarbital.


CONTROL
PENTOBARBITAL
A
B
C
10 mV
10 ms
20 mV
20 ms

enhancement of the $GABA_d$ response, which was accompanied by an inhibition of responses to afferent stimulation. Pentobarbital appeared to have negative effects on the other responses to GABA, yet these data could be misleading, since a large potentiation of the $GABA_d$ response could mask effects on the $GABA_s$ response or the late hyperpolarization.

2. DIAZEPAM

The effects of several different concentrations of diazepam were tested on seven cells (Table IV). At the lowest concentration (1 uM; n=1; Table IV, cell 1), diazepam had no effect. An intermediate dose (10 uM) of diazepam was tested on four cells. In the first cell a $GABA_d$ response was monitored, and a small increase in conductance was observed (Table IV, cell 2). In three other cells $GABA_{s-d}$ responses, each followed by a late hyperpolarization, were elicited. There was an increase in the amplitude and conductance change of the $GABA_s$ response and late hyperpolarization in one cell (Figure 17A; Table IV, cell 3), no changes in amplitude but an enhanced conductance change in a second cell (Table IV, cell 5), and mixed effects in the third cell (Table IV, cell 4).

High doses of diazepam (50 uM and 500 uM) were also tested. In one of these cells, (Table IV, cell 6), the response to GABA was enhanced, but 1) the RMP was negative to -70 mV, and 2) the reversal potential was not measured. Therefore, it was not possible to determine what response type was affected. In the second cell, a $GABA_{s-d}$ response followed by a late hyperpolarization was elicited (Figure 17B; Table IV, cell 7). There was an increase in the amplitude and change in conductance of the $GABA_s$ response (for some doses of GABA), and the late hyperpolarization (all doses of GABA), but there was no effect on the $GABA_d$ response.

Figure 17. Diazepam increases the amplitude and conductance change during $GABA_s$ responses and the late hyperpolarization. MP=membrane potential at which the GABA responses were elicited.

A. GABA (50 ms, 30 psi, at the arrow) was ejected less than 50 um from the soma. At -60 mV (1) and -70 mV (2 and 3) responses to GABA are shown in control (left) and following bath-application of 10 uM diazepam for 5 minutes (right). Dotted line in (2) indicates RMP. In (3) fixed amplitude hyperpolarizing current pulses (0.25 nA, 150 ms) were delivered through the recording electrode during the GABA response. Cell location: Layer V. RMP=-70 mV.

B. In another cell in Layer V, GABA (100 ms, 35 psi) was ejected 100 um towards the WM from the soma. On the left, a $GABA_s$ response followed by a late hyperpolarization was elicited in control. On the right, the response to the same pressure pulse of GABA is shown during bath-application of 500 uM diazepam. Both recordings were taken at -66 mV membrane potential. RMP=-73 mV.

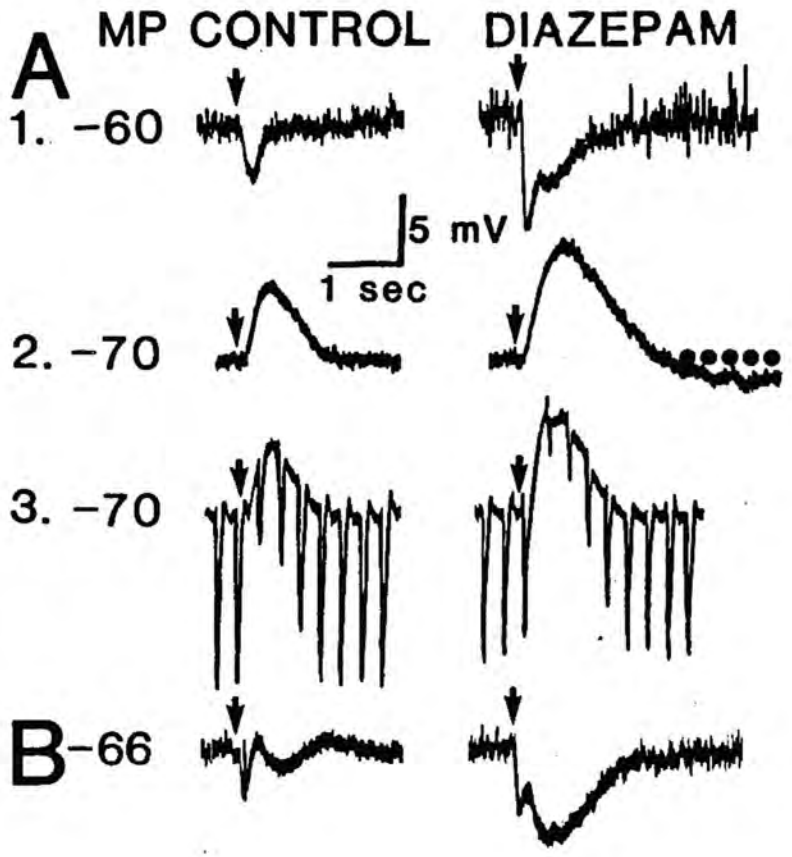

A
MP
CONTROL
DIAZEPAM
1. −60
5 mV
1 sec
2. −70
3. −70
B
−66

TABLE IV

Effects of Benzodiazepines on GABA Responses

CELL	[DZP]	RESPONSE TYPE	G_S	G_D	LATE HYPERPOLARIZATION	COND
1	1.0 uM	G_{s-d-LH}	NC	NC	NC	NT
2	10	G_d		NC		+
3	10	G_{s-d-LH}	+	+,-	+	+
4	10	G_{s-d-LH}	-	+,-	NC,+	+,-,NC
5	10	G_{s-d-LH}	NC	NC	NC	+
6	50	G_d or G_{s-d}	+	+		+
7	500	G_{s-d-LH}	NC,+	NC	+	NT
8	25(CLON)	G_{s-d-LH}	NC	NC	NC	-

Responses listed in the same row were obtained from the same cell.

Experimental procedure:

First, GABA responses were elicited by ejection of GABA at the soma or on the proximal dendrites. The pressure pipette was not moved for the remainder of the experiment. Several doses of GABA were ejected, and these responses were repeated to ensure their reproducibility. Next, drug was bath-applied. During bath-application, the same doses of GABA were tested to monitor possible changes in the responses to GABA.

KEY

DZP = diazepam
CLON = clonazepam
COND = conductance increase during the GABA response
+ = increased
- = decreased
+,- = increase in responses elicited by some doses of GABA, decrease in responses elicited by other doses
NC = no change
NT = not tested
G_{s-d-LH} = $GABA_{s-d}$ response followed by a late hyperpolarization

Figure 18. Diazepam enhances hyperpolarizing potentials following orthodromic stimulation.

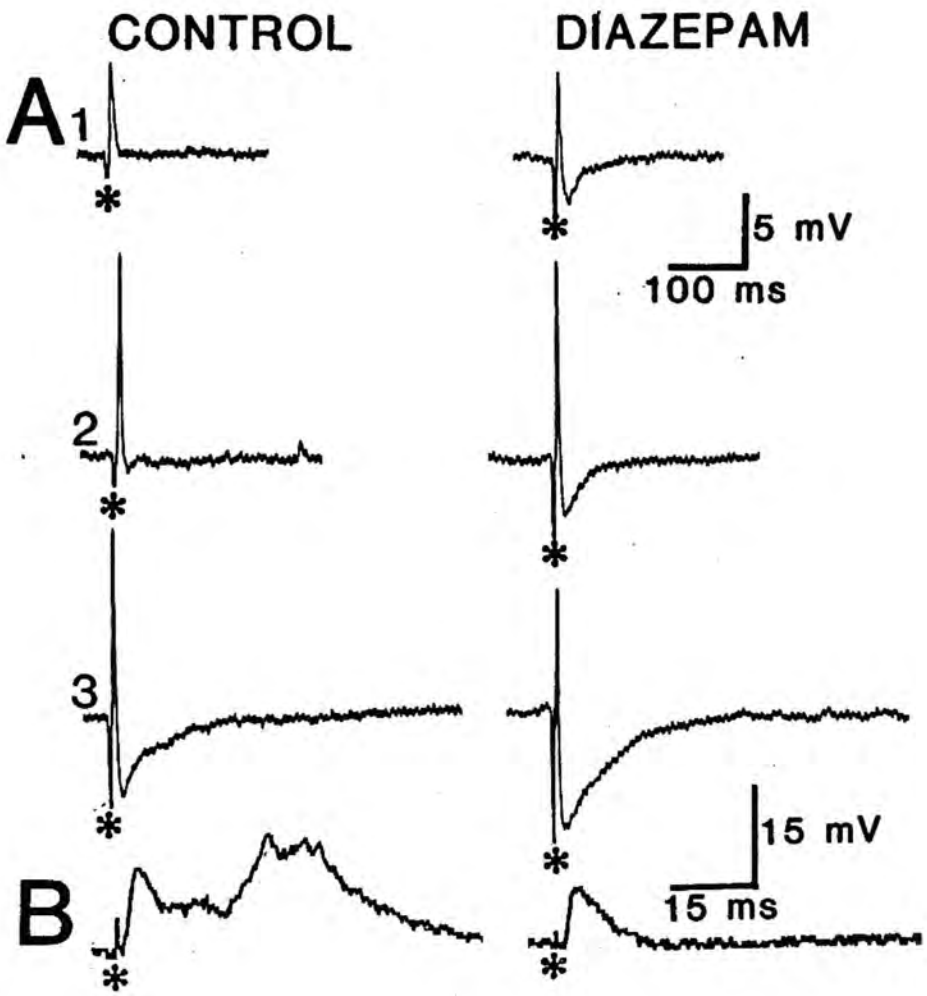
A. Responses to WM stimulation are shown. Asterisks indicate stimulus artifact. Cell location: Layer V. RMP=-68 mV. Action potentials were truncated by the chart recorder.

CONTROL: 1) A 0.075 mA stimulus evoked an EPSP. 2) A 0.1 mA stimulus elicited a larger EPSP. 3) At twice threshold stimulus strength an action potential was elicited, followed by an AHP. All stimulus durations were 100 us.

DIAZEPAM: After 50 uM diazepam was bath-applied for 46 minutes, the same stimuli elicted larger hyperpolarizations after the EPSPs or the action potential.

B. CONTROL: In another Layer V cell, a 0.25 mA, 100 us stimulus to the WM produced a multiphasic, long-lasting depolarization. RMP=-72 mV.

DIAZEPAM: During bath-application of diazepam (10 uM), the initial phase of the EPSP was depressed and the late phases of the depolarization were blocked.


CONTROL
DIAZEPAM
A
1
2
3
B
5 mV
100 ms
15 mV
15 ms

The effect of bath-application of the more potent benzodiazepine clonazepam (25 uM) was observed in a seventh cell. In this cell there was no change in the amplitude of the GABA response (Table IV), but there was a very small decrease in conductance.

There were very few changes in physiological properties and responses following bath-application of either benzodiazepine. For example, there were no changes in the RMP, and in only one cell was there a change in R_{in}. However, in the two cells that produced IPSPs and AHPs following orthodromic stimulation, these were greatly enhanced after diazepam was applied (Figure 18A). In one other cell, there was an increase in the stimulus strength required to reach threshold, and a decrease in the amplitude of the late phases of the EPSP (Figure 18B). The decrease in the late phases may be due to the enhancement of an underlying IPSP by diazepam.

In summary, the effects of diazepam varied, but some generalizations can be made. First, there was an enhancement of the conductance change during GABA responses. Second, there was an increase in amplitude of late hyperpolarization in many cells. In addition, aspects of cell physiology that may reflect the level of inhibition, such as the IPSP and AHP, were enhanced.

3. BICUCULLINE METHIODIDE

The effects of the GABA antagonist bicuculline methiodide (BMI) were examined by monitoring responses to pressure-applied GABA before and during bath-application of BMI. In 5 of 5 cells, the GABA responses were decreased or blocked during bath-application of 50 uM BMI. The onset of BMI's effects were extremely rapid, requiring 5-10 minutes of perfusion. It appeared that $GABA_d$ responses were preferentially affected, because,

Figure 19. Bicuculline methiodide depresses the $GABA_s$ response and blocks the $GABA_d$ response and late hyperpolarization.

A. CONTROL

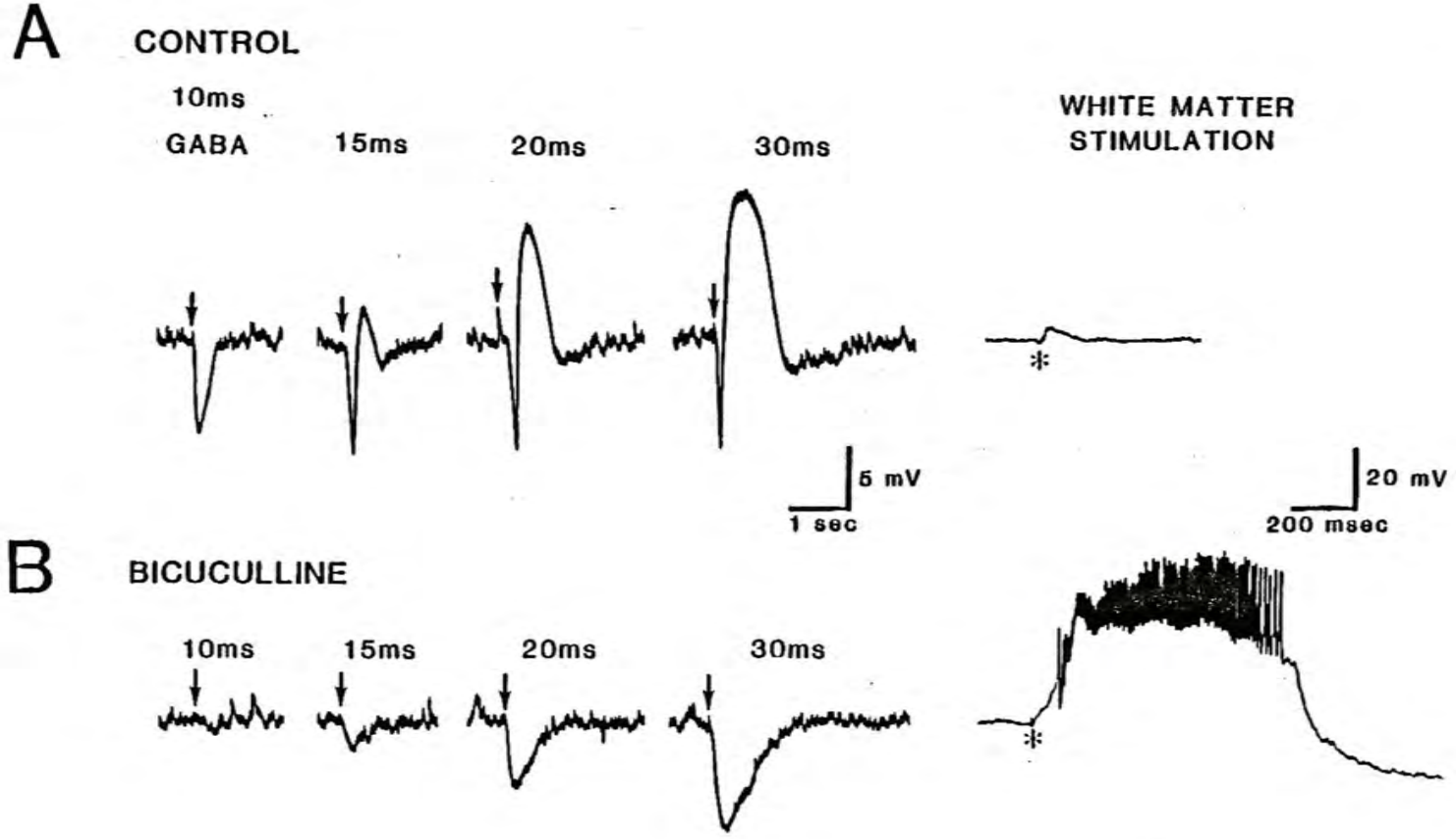
Left: Increasing amounts of GABA were pressure-applied (9 psi, at the arrows) less that 50 um from the soma.

Right: Stimulation of the WM (0.2 mA, 200 us, at the asterisk) produced an EPSP.

B. BICUCULLINE: Responses to the same pressure pulses of GABA and to the same stimulus as in control are shown after 50 uM bicuculline methiodide was bath-applied for 4 minutes.

Left: $GABA_d$ responses and the late hyperpolarization were blocked, and $GABA_s$ responses were blocked at low doses.

Right: The same stimulus which evoked an EPSP in control elicited a burst of action potentials after bath-application of bicuculline. Action potentials were truncated by the chart recorder. Cell location: Layer V. RMP=-67 mV.


A
CONTROL
10ms
GABA
15ms
20ms
30ms
WHITE MATTER
STIMULATION
5 mV
1 sec
20 mV
200 msec
B
BICUCULLINE
10ms
15ms
20ms
30ms

when $GABA_d$ responses were blocked, $GABA_s$ responses were unaffected or only slightly inhibited (Figure 19). In two of these five cells, a late hyperpolarization occurred after the $GABA_{s-d}$ response and was suppressed by BMI in both cases (Figure 19). All effects on GABA responses partially reversed in the two cases where impalements were maintained for a sufficient wash period (over 90 minutes).

In one other cell a lower concentration of BMI (0.1 uM) was bath-applied. After 15 minutes the $GABA_d$ response was blocked and the $GABA_s$ phase was depressed. The effects were evident simultaneously, which suggests that the effects on the $GABA_s$ and $GABA_d$ response can not be differentiated, even when low doses of BMI are used. However, use of nanomolar concentrations, and slower perfusion rates, would be useful future experiments.

BMI also had effects on the responses of the cell to orthodromic stimulation, which coincided temporally with the effects on GABA responses. Orthodromic stimulation which produced no response or a subthreshold response prior to addition of BMI, produced a burst of action potentials on a large depolarization during bath-application of BMI (Figure 19). In three cells both spontaneous and evoked bursts occurred. For individual cells and upon comparison of all cells, spontaneous and evoked bursts had a stereotyped form. They consisted of numerous action potentials, some of very small amplitude, firing at a fast frequency on a large and long-lasting depolarization (up to 40 mV, 2 sec). This depolarization was quite similar to the paroxysmal depolarizAtion shift observed by others in epileptiform cortical tissue (Matsumoto and Ajmone-Marsan, 1964a and b), and was followed by a large AHP.

Four of the six cells depolarized during bath-application of BMI.

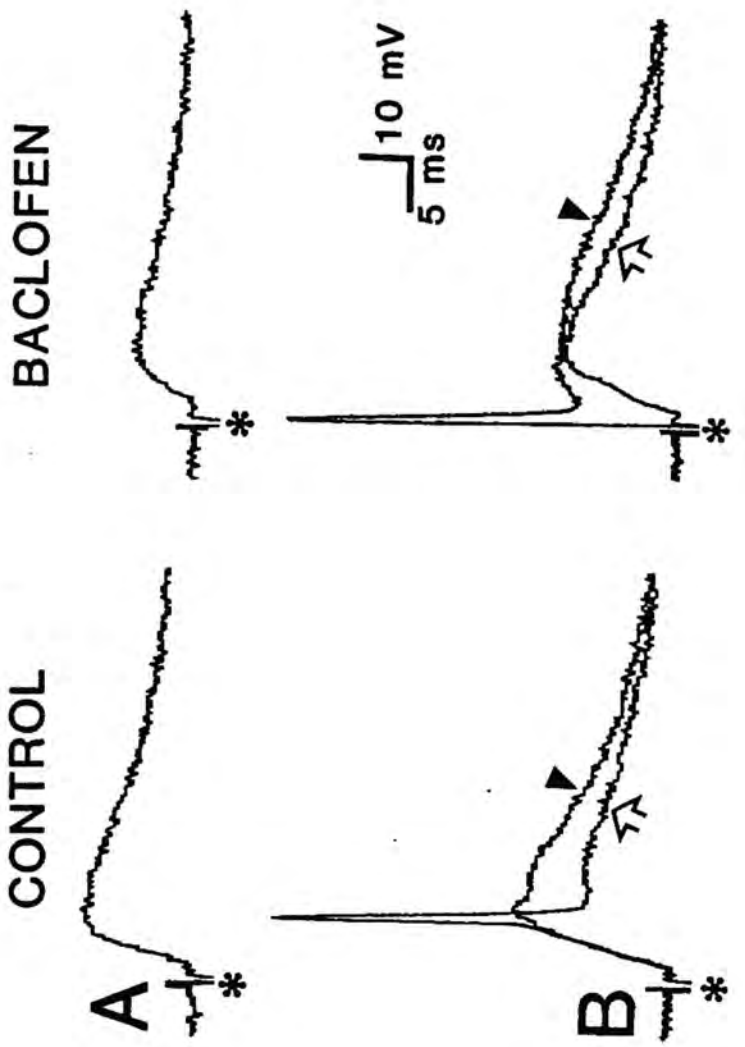
These depolarizations were very large and occurred quickly; the RMPs depolarized 10 to 60 mV in a few minutes. The depolarization occurred shortly after the GABA responses were affected, and after the epileptiform bursts were first noticed. R_{in} was measured during the depolarization in one cell, and it had fallen to zero. Despite the magnitude of these changes, in every case the effects on the RMP and R_{in} reversed within minutes of perfusion with control buffer. This contrasted to the effects on GABA responses and responses to orthodromic stimulation, which took hours to reverse. In the two cells which did not spontaneously depolarize during bath-application of BMI, there was no effect of BMI on the RMP, R_{in}, the responses to stimulation, or the responses to depolarizing current pulses. The subthreshold response of one of the six cells was an IPSP. In that cell the IPSP and the $GABA_s$ response were inhibited at the same time by bath-application of BMI. This common sensitivity to BMI is another example of the similarity of these two responses.

Thus, BMI was found to suppress GABA responses, as is consistent with the antagonist action of this drug. The effects of BMI on the late hyperpolarization should be interpreted with caution, since only two cells with late hyperpolarizations were tested, and these responses were very small and therefore difficult to measure. Simultaneous to the inhibition of GABA responses, many signs of epileptiform activity occurred in these cells.

4. BACLOFEN

Bath-application of the $GABA_B$ agonist (-)-baclofen (100 uM, n=4) had no effect on responses to pressure-applied GABA. However, bath-applied baclofen did affect the membrane potential, R_{in}, and

Figure 20. Baclofen inhibits responses to orthodromic stimulation.
A. LEFT: An EPSP was elicited by stimulation of the WM (0.2 mA, 200 us, at the asterisk).
RIGHT: During bath-application of baclofen (100 uM, for 7 minutes) the EPSP produced by the same stimulus was reduced in amplitude.
B. LEFT: In the same cell, stimulation at threshold (0.28 mA, 200 us) elicited an EPSP and an orthodromic action potential in control. The arrowhead indicates the EPSP, and the open arrow points to the action potential.
RIGHT: During bath-application of baclofen, the stimulus strength to reach threshold was increased to 0.83 mA, 200 us. Only antidromic action potentials could be elicited. Cell location: Layer V. RMP=-72 mV in control, -80 mV when the traces during bath-application of baclofen were recorded.


CONTROL
BACLOFEN
A
B
10 mV
5 ms

responses of these cells to stimulation. The RMP became more negative by 2-5 mV, and the R_{in} decreased greatly. EPSPs were depressed and the stimulus intensity required to reach threshold increased (Figure 20). In two cells orthodromic action potentials could no longer be evoked, even at stimulus strengths which were 10 times those used in control to evoke threshold spikes. Antidromic action potentials, however, could be elicited at high stimulus strengths in these cells (Figure 20). In three of the four cells the number of action potentials elicited by a depolarizing pulse decreased, as did the AHP following the pulse.

To examine the effects of baclofen in more detail, baclofen was pressure-applied to the soma and proximal dendrites with various concentrations in the pressure pipette (Table V). Baclofen responses could not be discriminated on the basis of the site of ejection (soma or dendrites). When the concentration in the pressure pipette was relatively low (1-10 uM) small hyperpolarizations were produced without a change in R_{in} (Figure 21). The amplitude and time course of these hyperpolarizations were usually less than those following bath-application.

Responses to higher doses of pressure-applied baclofen (100 uM- 1 mM) were similar to the effects of bath-applied baclofen. An example is shown in Figure 22. A pressure pipette (100 uM baclofen in 0.9% NaCl) was placed on the surface of the slice 100 um towards the PIA from the soma. The cell immediately hyperpolarized from -60 to -70 mV, and the pipette was removed. The R_{in} was tested and had decreased to half the control value. The resistance was less than that observed prior to the use of the baclofen pipette, when R_{in} was tested at membrane potentials which were artificially hyperpolarized by injection of DC current. In this experiment, synaptic transmission was blocked by prior bath-application

Figure 21. Effects of low doses of pressure-applied baclofen. Baclofen (10 uM in 0.9% NaCl) was ejected 150 um lateral to the soma (1.5 sec, 10 psi, indicated by the solid bar). A hyperpolarization was produced without a change in R_{in}. During the response fixed amplitude hyperpolarizing current pulses (0.5 nA, 50 ms) were given. The bracket surrounds action potentials, which were truncated by the chart recorder. RMP=-60 mV.

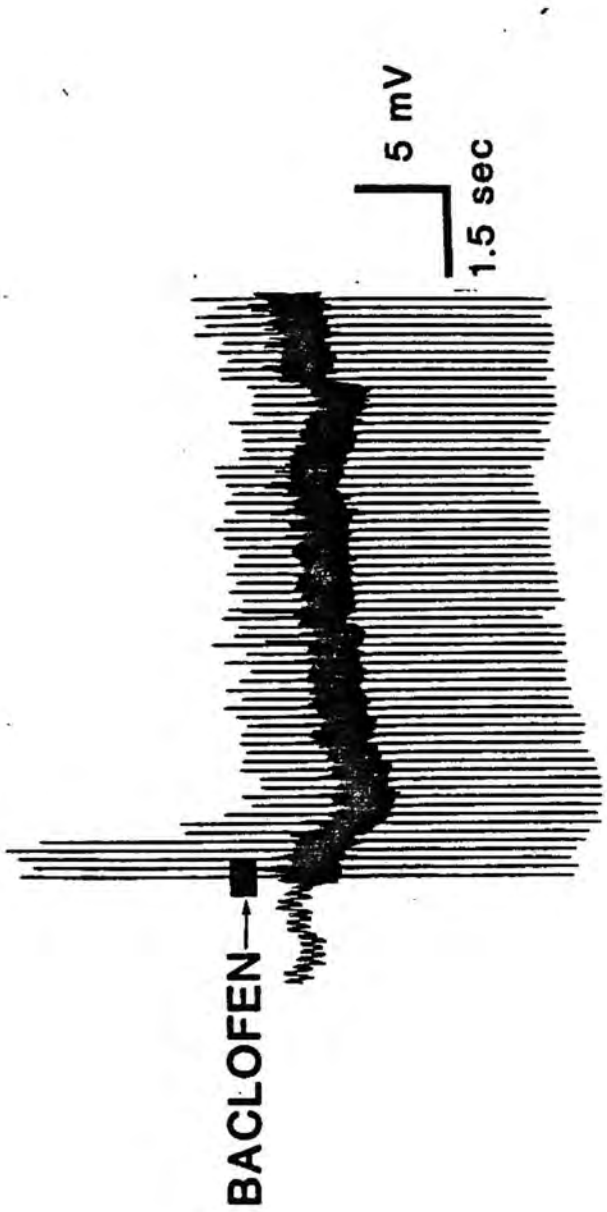

5 mV
1.5 sec
BACLOFEN

Figure 22. Baclofen produces a hyperpolarization and a decrease in input resistance, in the presence of tetrodotoxin.

CONTROL: Synaptic transmission was blocked by bath-application of 1 uM tetrodotoxin.

Top: The RMP of this Layer V cell was -60 mV. Potentials negative to -60 mV are below the trace; potentials positive to -60 mV are above it.

Bottom: The response to a hyperpolarizing current pulse (0.25 nA, 150 ms) is shown. R_{in}=40 megohms.

BACLOFEN: A pipette filled with (-)-baclofen (100 uM in 0.9% NaCl, at the arrow) was placed 100 um from the soma towards the pia.

Top: Immediately the membrane potential hyperpolarized 10 mV. The baclofen pipette was removed following the hyperpolarization.

Bottom: The R_{in} decreased from 40 to 20 megohms. This decrease was more than the decrease observed when the cell was hyperpolarized 10 mV in control.

RECOVERY:

Top: The membrane potential returned to the control value in 20 min.

Bottom: The R_{in} returned to control after 12 min.

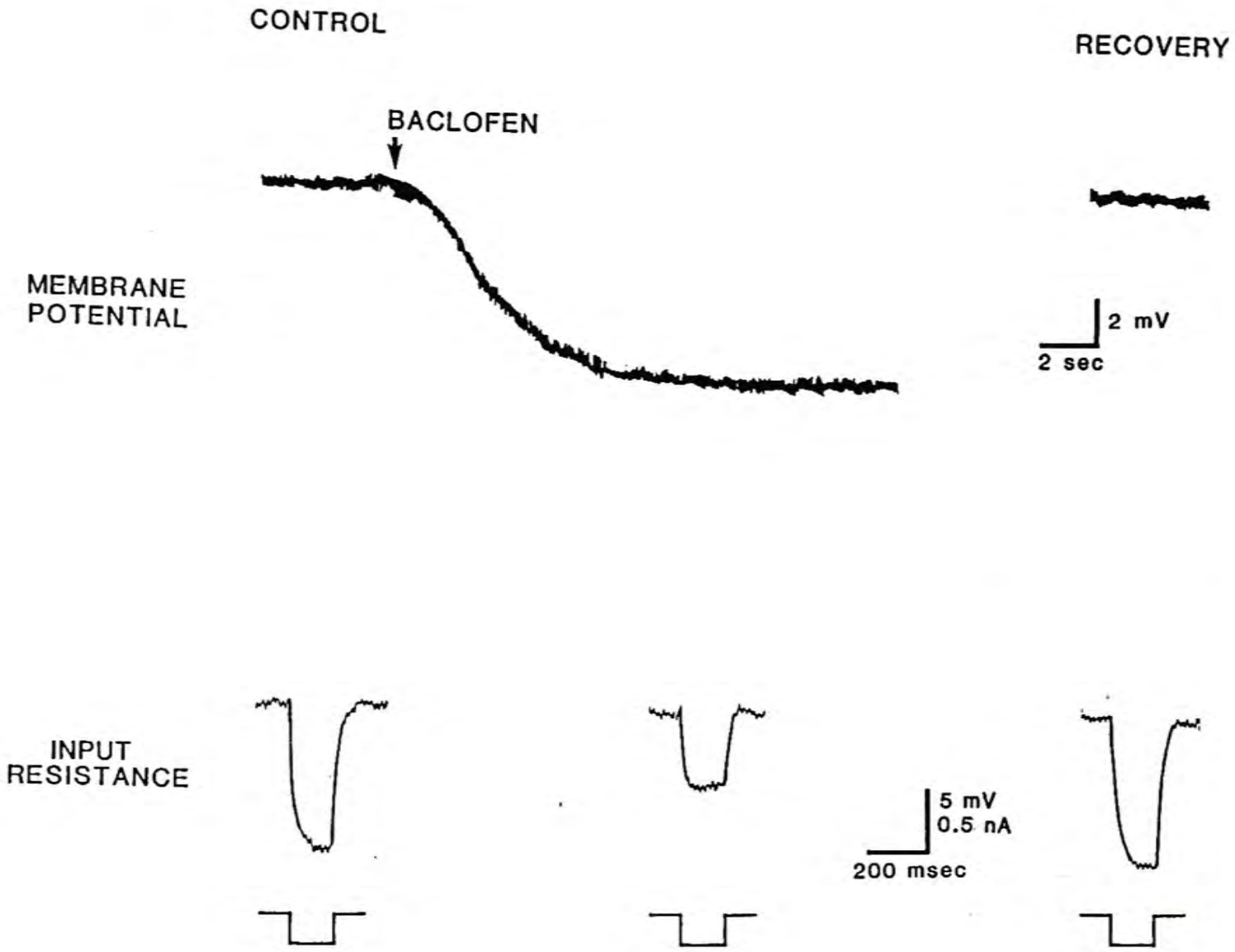

CONTROL
RECOVERY
BACLOFEN
MEMBRANE POTENTIAL
2 mV
2 sec
INPUT RESISTANCE
5 mV
0.5 nA
200 msec

TABLE V

Effects of Pressure-applied Baclofen

CELL	CONC	HYPER	HYPER + R_{in}	DEPOL-HYPER	NO RESPONSE
1	1 uM	PD			DD
2	1 uM	PD		PD	
3	10 uM	S			
4	10 uM	PD			
5	100 uM		S		
6	1 mM				S
7	1 mM	PD	DD		
8	1 mM				PD

Responses listed in the same row were obtained from the same cell.

S = baclofen was applied less than 50 um from the soma.

PD = baclofen was ejected in the proximal dendrites (50-150 um from the soma).

DD = baclofen was ejected in the distal dendrites (over 150 um from the soma).

of TTX. Therefore, the hyperpolarization and decrease in R_{in} were probably mediated by postsynaptic processes. Using the method of Ginsborg (1973; see Materials and Methods), the reversal potential of the baclofen response was calculated to be -90 mV. It was not possible to do this calculation in other cells.

When high doses were pressure-applied, and for all bath application experiments, the effects of baclofen on the RMP, R_{in}, and responses to current pulses were all reversible. However, the time course of reversal was very different. The effects on the R_{in} reversed relatively quickly, whereas the effects on the RMP took much longer to reverse. For example, the R_{in} of the cell shown in Figure 22 recovered in 12 minutes, but the RMP took 20 minutes to return to -60 mV.

In summary, baclofen did not affect responses to pressure-applied GABA, but did affect visual cortical cells in several ways. The RMP hyperpolarized, and, in many cases, there was a decrease in R_{in}. In addition, baclofen depressed responses to orthodromic stimulation.

ION SUBSTITUTION EXPERIMENTS

To determine if chloride, potassium, calcium, or sodium ions might be involved in the responses to GABA, a series of ion substitution experiments were performed. In most cases, the experiments were designed to alter the concentration gradient of a specific ion, while monitoring the responses to GABA at a fixed site of ejection.

1. CHLORIDE

In almost all areas of the mammalian brain, chloride ions mediate responses to GABA (Nistri and Constanti, 1979; Enna and Gallagher, 1983;

Simmonds, 1984). Therefore, the first experiments focused on whether chloride ions were involved in the responses to GABA of visual cortical neurons.

Use of recording electrodes filled with 3 M potassium chloride was a relatively simple way to examine the chloride-dependence of GABA responses, since these electrodes are generally easier to use for intracellular recording that potassium methylsulphate-filled electrodes. The high concentration of chloride ions in the electrode diffuse into the cell, and this reverses the concentration gradient for chloride.

Data were obtained from five cells using recording electrodes filled with 3 M potassium chloride. Under these conditions, responses to GABA ejection at the soma were depolarizing, even when the membrane potential was relatively depolarized (positive to -70 mV; Figure 23). This was a sharp contrast to the typical response to ejection of GABA near the soma when recording with potassum methylsulphate-filled electrodes, which was a hyperpolarization at membrane potentials positive to -70 mV. In four cells that were impaled with KCl electrodes, reversal potentials of the $GABA_s$ response were determined. The mean reversal potential of these responses was -49.2 $\pm$ 4.6 mV (Figure 23A), which was significantly different from the mean reversal potential of the $GABA_s$ response calculated from recordings with potassium methylsulphate-filled electrodes (-71.7 $\pm$ 1.1 mV; t-test, $p=<0.05$).

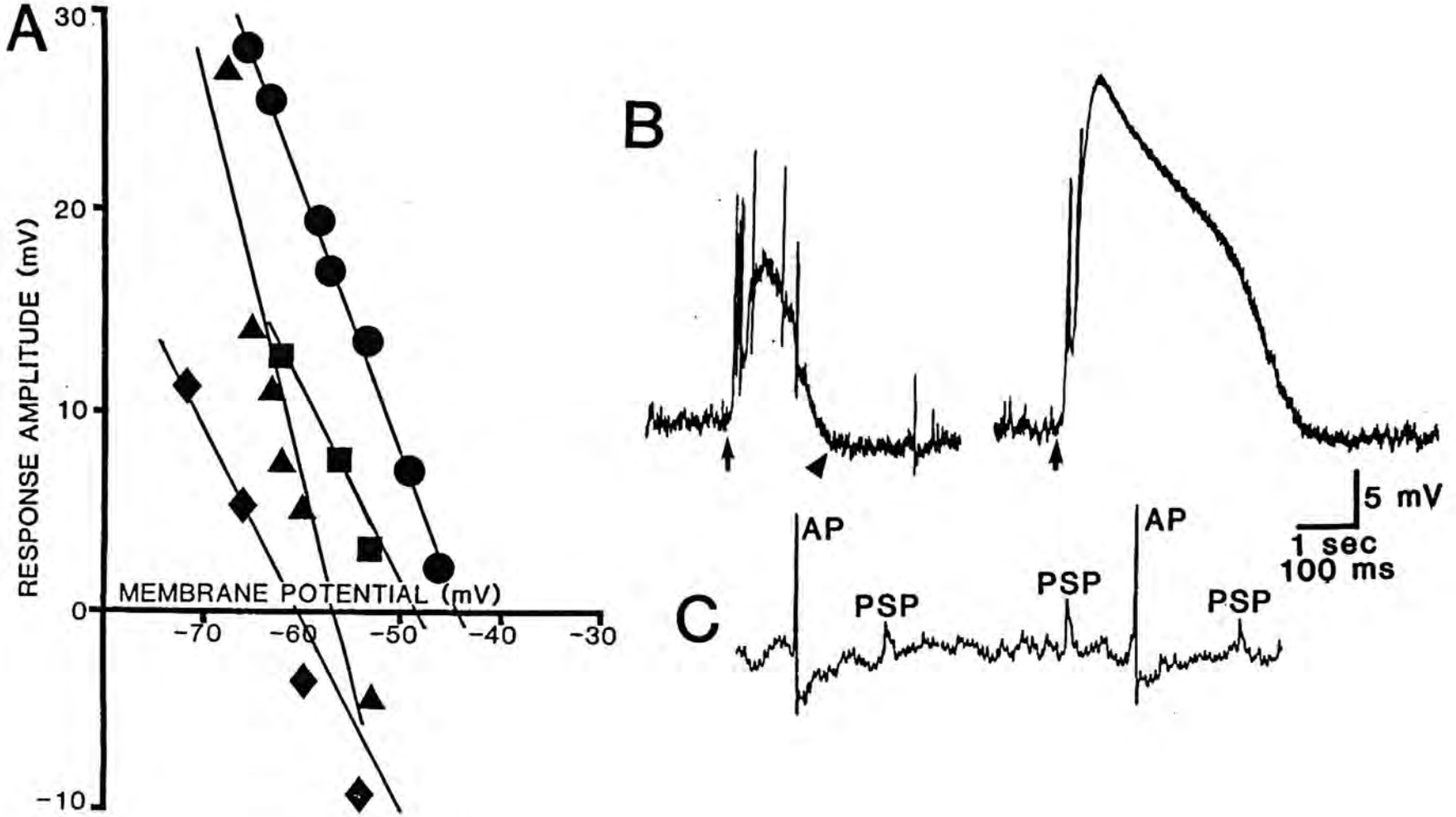
In four cells potassium chloride-filled electrodes were used to record dendritic responses elicited by ejection of GABA 100-250 um from the soma. A total of 7 sites of ejection were made on dendrites in these four cells. In two cells, the mean reversal potentials for all dendritic applications were more positive than those obtained with potassium methylsulphate-filled electrodes (KCl: -36.0, -43.4; KCH_3SO_4: -49.8 mV).

Figure 23. Change in reversal potential of the $GABA_s$ response of cells recorded with KCl-filled electrodes.

A. Reversal potentials of the GABA response at the soma were calculated in four Layer V cells impaled with KCl-filled electrodes. Each symbol represents data from a different cell. Lines were calculated by least squares regression analysis. The intersections of the lines with the x-axis are the reversal potentials of these cells.

B. GABA was ejected at the soma, and responses were recorded with KCl-filled electrodes. Pulse duration was increased from left (20 ms) to right (50 ms), and pressure was constant (35 psi). The arrowhead points to a late hyperpolarization following the depolarization of the response on the left. The late hyperpolarization did not follow the depolarization produced by the larger dose of GABA. It is possible that the depolarization was large and long-lasting enough to hide an underlying late hyperpolarization. RMP=-59 mV.

C. A segment of the membrane potential from the same cell as in B exemplifies the large amount of spontaneous activity. AP = spontaneous action potential. PSP = spontaneous postsynaptic potential. Action potentials were truncated by the chart recorder.


A
30
20
10
0
−10
RESPONSE AMPLITUDE (mV)
MEMBRANE POTENTIAL (mV)
−70
−60
−50
−40
−30
B
5 mV
1 sec
100 ms
C
AP
PSP
AP
PSP
PSP

However, the mean reversal potentials of the responses to GABA of the other two cells were similar to those when potassium methylsulphate-filled electrodes were used (-46.0, -49.0).

The late hyperpolarization did not appear to change when potassium chloride electrodes were used. A late hyperpolarization followed the depolarizations of three cells, an example of which is shown in Figure 23B (indicated by the arrowhead). The mean reversal potential for these responses was -75.3 $\pm$ 2.1 mV, which is not significantly different from that derived from data recorded with potassium methylsulphate-filled electrodes (t-test, $p>0.05$).

Subthreshold synaptic responses were monophasic and depolarizing in all cells recorded with potasium chloride-filled electrodes. These could have represented EPSPs, but they could also have represented inverted, chloride-dependent, IPSPs. This problem was investigated further in one cell, where the reversal potential of the synaptic response was determined. It was found that the PSP reversed at a potential similar to the $GABA_s$ response in that cell (PSP:-40.4 mV; $GABA_s$ response:-42.2 mV), and much different from the EPSP in other cells (-28.4 $\pm$ 3.2 mV, n=5). This suggests that the PSP was an inverted IPSP. The fact that both the IPSP and the $GABA_s$ response may be chloride-dependent further supports the hypothesis that GABA may be the transmitter which mediates the IPSP.

The RMP and R_{in} of these cells were similar to cells impaled with potassium methylsulphate-filled electrodes. However, there were some differences. First, there was much more spontaneous activity, in the form of spontaneous postsynaptic potentials, which were always depolarizing, and action potentials (Figure 23B and 23C). There was much more noise in the membrane potential trace; this may be a reflection of an increase in

synaptic activity (Figure 23B and 23C). Finally, more action potentials fired during depolarizing current pulses. Instead of the typical 4-7 action potentials during a 1.0 nA, 150 ms pulse, over 10 action potentials fired. There were no differences in R_{in} or RMP, however. These changes may point out processes that are chloride-dependent, and, since the data indicate a chloride-dependence of the $GABA_s$ response, they might reflect processes that are mediated by GABA.

Thus, chloride ions appear to be important to the $GABA_s$ and $GABA_d$ responses, but not the late hyperpolarization. Since chloride appears to underly many GABA responses, and alteration of the chloride gradient markedly affected the level of spontaneous activity, the results of these experiments suggest that GABA plays an important role in the control of visual cortical activity. This is consistent with the experiments using bicuculline methiodide, which demonstrated that when GABA responses were suppressed, epileptiform activity occurred.

2. POTASSIUM

Although the GABA responses are chloride-mediated in most cases, there is a precedent for potassium-dependent responses as well. In hippocampus, it has been suggested that there may be a potassium component to the chloride-dependent response to GABA ejection on the soma (Eccles et al., 1964; Lux, 1974). In some non-mammalian preparations GABA is known to activate potassium conductances (Dudel, 1979; Nistri and Constanti, 1979). Therefore, it was of interest to determine what role potassium ions played in the generation of GABA responses.

Experiments were designed to change the gradient for potassium ions by bath-application of a solution containing a concentration of potassium three times that of control (3 mM vs. 9 mM). The amplitude and

Figure 24. Effects of bath-application of buffer containing 9 mM potassium.

All data were recorded from the same cell in Layer V. RMP=-71 mV. Action potentials were truncated by the chart recorder. Asterisk indicates stimulus artifact.

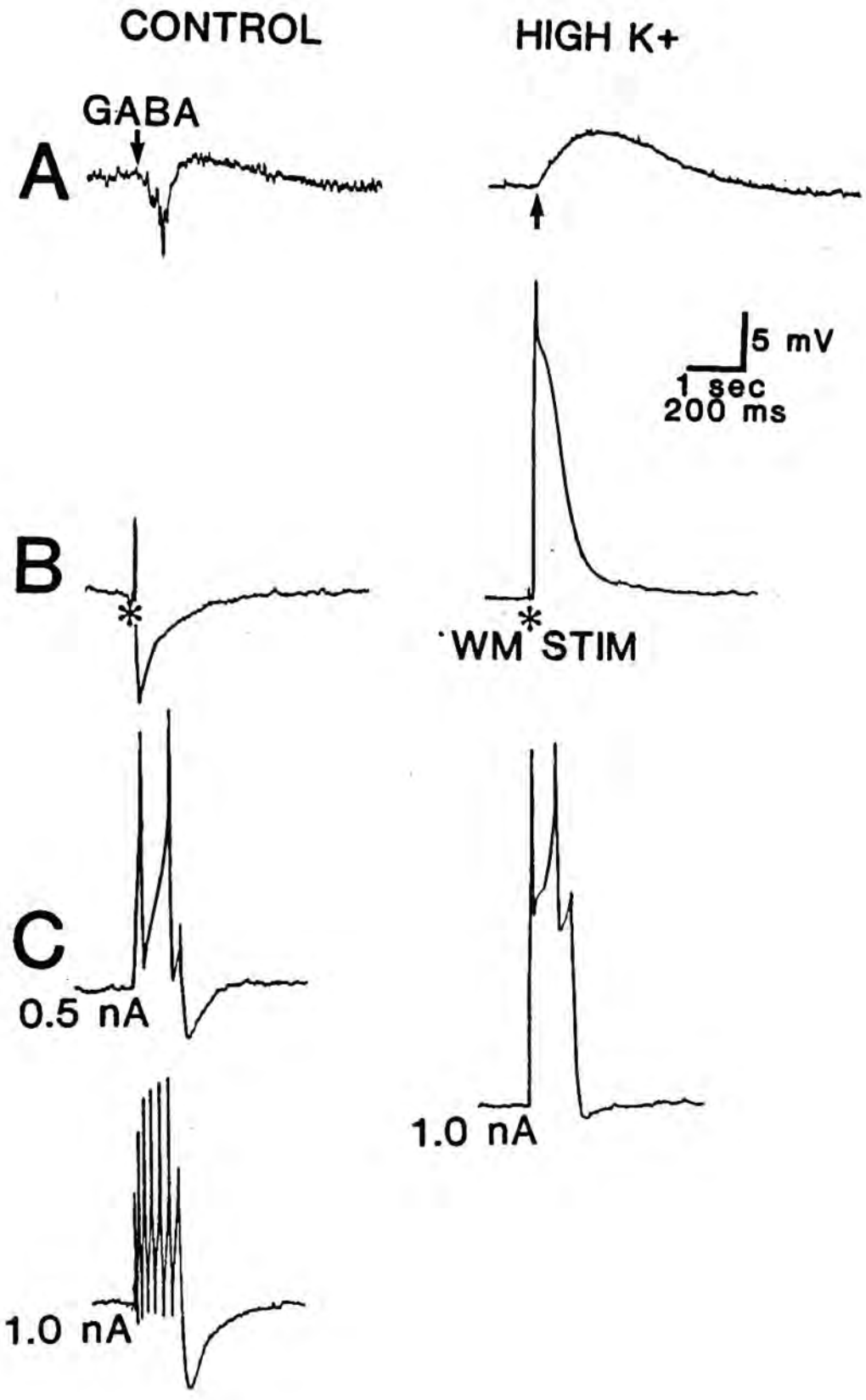
CONTROL: The slice was perfused with Krebs-Ringer buffer containing 3 mM potassium.

HIGH K+: The same slice was perfused with Krebs-Ringer buffer containing 9 mM potassium.

A. A 25 ms pulse of GABA (38 psi) was ejected 75 um towards the PIA from the soma (at the arrow), and was recorded at -62 mV. In control, a $GABA_{s-d}$ response was followed by a late hyperpolarization. Following bath-application of high-potassium buffer for 18 minutes, the same pulse of GABA produced a pure depolarization. The line indicates the RMP.

B. Stimulation of the WM (0.15 mA, 100 us; at the asterisk) evoked an action potential, followed by an AHP in control. In high-potassium buffer the same stimulus also elicited one action potential but the AHP completely reversed polarity. Action potentials were truncated by the chart recorder.

C. Responses to depolarizing current pulses (150 ms duration) are shown. In control, a 0.5 nA pulse produced two action potentials, and was followed by an AHP. A 1.0 nA pulse produced six action potentials, and was also followed by an AHP. During perfusion with high potassium buffer, a 1.0 nA current pulse produced two action potentials and the AHP was almost completely blocked. In B and C responses were recorded at -71 mV.


CONTROL
HIGH K+
GABA
A
5 mV
1 sec
200 ms
B
WM STIM
C
0.5 nA
1.0 nA
1.0 nA

reversal potential of GABA responses of all four cells tested were affected by the high potassium solution (Figure 24A).

A pure $GABA_d$ response was obtained in one cell. The reversal potential of this response shifted to a less negative potential by 5 mV. In two other cells, a $GABA_{s-d}$ response was monitored. The $GABA_s$ response reversed polarity and its reversal potential became much less negative (17 mV shift in the first cell and 27 mV in the second). The $GABA_d$ response increased in amplitude, and its reversal potential also became less negative (13 mV shift in the first cell and 20 mV in the other). The Nernst equation predicts a 28 mV change for a three-fold change in potassium concentration, at 35°C (Katz, 1966). The changes in reversal potential were not Nernstian in the first cell, but they were in the second cell. Therefore, potassium may be involved in $GABA_s$ and $GABA_d$ responses, but is probably not the sole ion involved. In the one cell tested, the effect of high potassium solution on the late hyperpolarization was dramatic. The reversal potential became more positive, and this change was Nernstian.

At the time that the GABA responses were affected, other aspects of cell physiology were also altered. These processes were those which are known to be potassium-dependent. For example, in three of four cells the RMP depolarized transiently 5-11 mV. This was not surprising, since the RMP of mammalian cells is highly dependent on this ion. (Ferriera and Marshall, 1985). In all cells there was a large drop in R_{in} (50-67% of control). In two cells, a decrease in the number of action potentials firing for a given depolarizing current pulse occurred (Figure 24C). The decrease in the number of action potentials firing may reflect a decrease in the potassium dependent process known as accommodation (Madison and Nicoll, 1984). This decrease could also be a consequence of the decrease

in R_{in}.

A calcium-dependent potassium current underlies the AHP following orthodromic stimulation (Barrett and Barrett, 1976; Krnjevic et al., 1978), and depolarizing pulses (Krnjevic et al., 1978; Hotson and Prince, 1980). All AHPs following orthodromic action potentials reversed polarity during bath-application of high potassium buffer (Figure 24B). Other changes in the responses to orthodromic stimulation also occurred. In two of four cells, threshold increased. In the two cells where IPSPs were elicited, they reversed polarity. The fact that both the IPSP and $GABA_s$ response were affected by the high potassium solution in the same way, is another example of the similarity between these two events.

When high-potassium buffer was bath-applied, the amplitude of the AHP following depolarizing pulses decreased or was blocked in three of four cells. (The fourth cell was the same cell as the one which did not depolarize in high-potassium buffer, and it is unclear why this cell was different from the other three cells.) The change in AHP amplitude occurred regardless of whether the AHP was compared to the AHP following the same amplitude pulse given in control, or if it was compared to the AHP following a pulse that produced the same number of action potentials in control (Figure 24C).

In summary, potassium ions may contribute to the $GABA_s$ and $GABA_d$ responses, but other ions are probably also involved. Preliminary results suggest that the late hyperpolarization is entirely potassium-dependent. In addition, these experiments demonstrate the similarity of visual cortical neurons with other central neurons, in that they share similar properties, and a similar potassium-dependence of those properties.

3. CALCIUM

There are several studies which suggest that calcium plays an important role in the actions of GABA, both at the $GABA_A$ receptor and the $GABA_B$ receptor (Bowery et al., 1980; Dunlap and Fischbach, 1981; Bowery et al., 1983; Majewska and Chuang, 1984). Therefore, experiments were undertaken to examine the possibility that calcium contributes to the GABA responses of visual cortical neurons.

Two approaches were used. In three cells a solution containing 0 mM $CaCl_2$ and 10 mM $MgCl_2$ was bath-applied. In a fourth cell $CaCl_2$ was replaced by equimolar $MnCl_2$, a calcium antagonist. In all four cells synaptic transmission was blocked. There was no change in RMP or R_{in}. There were no consistent changes in the responses to depolarizing current pulses. At the same time there was no change or a slight decrease in the amplitudes of the $GABA_{s-d}$ (n=2) or $GABA_d$ (n=2) responses. There was no change in the reversal potentials of the GABA responses. The results suggest that calcium does not mediate any of the responses to GABA.

4. SODIUM

Sodium ions are involved in the synaptic potentials produced by a number of neurotransmitters (Katz, 1966; Shepherd, 1979). Thus some experiments were devoted to establishing whether sodium ions mediate GABA responses.

Tetrodotoxin (TTX) is known to block voltage-dependent sodium channels (Narahashi et al., 1964). Instead of changing sodium concentrations, which entails complex substitutions of the Krebs-Ringer solution, the effects of sodium on GABA responses was examined by bath-applying TTX (1 uM, n=3). When synaptic transmission was blocked and no action potentials could be elicited by depolarizing current pulses, there was no change in the GABA responses elicited in any of the

DISCUSSION

RESPONSES TO SYNAPTIC STIMULATION AND INTRACELLULAR CURRENT INJECTION

The biophysical properties and responses of Layer V neocortical neurons to synaptic stimulation and intracellular current injection have been recorded in a slice preparation of rat visual cortex. The results were similar to several recent in vitro studies of other neocortical areas (Connors et al., 1982; Vogt and Gorman, 1982; Flatman et al., 1984; McCormick et. al., 1986).

Intracellular injection of the fluorescent dye Lucifer yellow allowed morphological identification of two distinct groups of cells: pyramidal and nonpyramidal cells. Despite morphological differences, there were very few physiological distinctions between these two groups of cells. Properties such as resting membrane potential, action potential amplitude, and input resistance were not significantly different. Responses to hyperpolarizing and depolarizing current pulses were not different either. There are, however, more detailed analyses that might have revealed more subtle differences. For example, McCormick and colleagues recently found that the action potentials of nonpyramidal cells have a much shorter duration and a sharper falling phase than those of pyramidal cells in anterior cingulate cortex (McCormick et al., 1985; McCormick and Prince, 1985).

Differences exist in the responses of visual cortical pyramidal cells and nonpyramidal cells to afferent stimulation. In response to stimulation of the PIA or WM, nonpyramidal cells typically produced a multiphasic, long-lasting depolarization following a subthreshold stimulus, and multiple action potentials at and above threshold. Pyramidal cells produced a shorter-lasting depolarization or a

depolarization followed by a hyperpolarization. It is important to point out that reversal potentials of synaptic responses were not calculated in all cells, and thus some of the depolarizations of cells with RMPs more negative than -70 mV could have been inverted IPSPs. Therefore, it may be possible to detect IPSPs in nonpyramidal cells in future studies.

The results demonstrate that pyramidal cells and nonpyramidal cells respond differently to synaptic stimulation, and this is consistent with studies that have shown differences in their synaptic connections (Parnavelas et al., 1977; Ribak, 1978; Peters, 1981; White, 1981; Somogyi et al., 1982; Wolff and Cronwall, 1982; Somogyi et al., 1984b). However, pyramidal and nonpyramidal cells appear to have similar responses to current injection and properties such as RMP and action potential amplitude, which indicates that they share some of the same intrinsic properties. This is one of the first attempts to correlate morphological characteristics with physiological properties of cortical neurons at the intracellular level. It provides a basis for further study of the physiological properties and functional roles of cells that differ morphologically, both in the neocortex, and in other areas of the brain as well.

GABA RESPONSES

There were three major findings concerning the effects of GABA on visual cortical neurons.

First, all neurons tested responded to GABA when it was applied within 300 um from the soma. The widespread effect of GABA in visual cortex is consistent with the general consensus that GABA is a major inhibitory transmitter in the brain (Eccles, 1964; Nistri and Constanti, 1979; Enna and Gallagher, 1983; Simmonds, 1984). However, the

possibility exists that some cell types were not impaled, and these cells might not have responded to GABA. In any case, these data suggest that a substantial number of GABA receptors exist and affect neurons throughout the visual cortex. The results contribute to the growing body of evidence that GABA plays a major role in visual cortical function (Curtis and Felix, 1971; Pettigrew and Daniels, 1973; Rose and Blakemore, 1974; Daniels and Pettigrew, 1975; Sillito, 1975a and b; Ito, 1976, Tsumoto et al., 1979; Sillito, 1984; Bagust et al., 1985).

The second major finding was that these cells shared a specific topographical pattern of GABA responsiveness. Generally, hyperpolarizations were produced when GABA was applied to the soma ($GABA_s$ response; mean reversal potential=-71.7 mV), and depolarizations were produced when GABA was applied to the dendrites ($GABA_d$ response; mean reversal potential=-49.3 mV). GABA ejected on proximal dendrites produced a combination of the two types of responses. In some cells, a third type of response, with a distinct reversal potential (mean reversal potential=-79.8 mV), followed the $GABA_s$ or $GABA_d$ response. This response was always hyperpolarizing, small in amplitude, and usually long-lasting. This response might have been elicited in all neurons, if sufficiently large quantities of GABA had been ejected, but doses of GABA were purposely kept small. It is also possible that $GABA_d$ responses masked some or all of this late hyperpolarization.

The third major finding was that these GABA responses were the same regardless of morphological differences. It was surprising to find that GABA had a uniform effect on cortical cells, since they are thought to have very different functions (Creutzfeldt, 1977; White, 1981; Evarts et al., 1984; Jones, 1984). The fact that all cells have similar responses to GABA makes the cortical GABAergic system seem quite simple

in design. Perhaps the expected complexity is provided by the additional factors that determine when and where GABA will be released, and therefore its ability to influence cell activity. For example, there are many different types of GABAergic interneurons in visual cortex, and their patterns of innervation are quite different (Szentagothai, 1973; White, 1981; Jones, 1984). Also, GABAergic cortical neurons contain different peptides which may be coreleased with GABA (Hendry et al., 1984; Somogyi et al., 1984b), and may underly complex modulation of GABA's actions.

THE ROLE OF GABA IN THE VISUAL CORTEX

GABA appears to have the ability to affect visual cortical neurons in different ways, and perhaps has more than one function in the visual cortex. One of these functions may be mediation of the hyperpolarizations (possibly IPSPs) recorded from some Layer V cells, namely pyramidal cells, following stimulation of the PIA or WM. There is a great deal of anatomical and biochemical data that support the hypothesis that GABA is a transmitter in the neocortex (Nistri and Constanti, 1979; Enna and Gallagher, 1983, Fagg and Foster, 1983; Gallagher and Shinnick-Gallagher, 1983). The results reported here further support this hypothesis. First, it has been found that pressure-application of GABA to the soma mimics the membrane potential change of the early phase of the IPSP. Second, the $GABA_s$ response and the early IPSP share a similar reversal potential, which indicates a common mechanism. Third, their effects are both inhibitory. Thus, the physiological relevance of the effects of GABA on cortical cell bodies may be that GABA is the neurotransmitter that mediates the early IPSP.

The effects of GABA on dendrites may be important to the

modulation of the various afferent inputs to the dendrites of cortical cells. For example, activation of GABAergic cells which synapse on the apical dendritic tree of pyramidal cells could inhibit the excitatory input to that dendritic tree. However, the inhibition of inputs to the apical dendritic tree might facilitate excitatory input to the basal dendritic trees. This might explain why, in some cases, spontaneous action potentials occur on the peaks of $GABA_d$ responses (Figure 7B3). Thus, the effect of GABA on dendrites could be inhibitory or excitatory.

An entirely different point of view is that dendritic GABA receptors are extrasynaptic. Extrasynaptic receptors have been found in several systems (Brown et al., 1981), and are suggested to occur on hippocampal pyramidal cells (Alger and Nicoll, 1982b), which respond to GABA in very similar ways to visual cortical cells. However, it is not necessary to postulate that extrasynaptic receptors exist to account for dendritic responses to GABA, since many laboratories have shown that GABAergic synapses are made on many dendritic elements throughout the visual cortex (Ribak, 1978; Peters, 1981; Peters et al., 1982; Somogyi et al., 1982; Wolff and Chronwall, 1982).

The functional relevance of the late hyperpolarization is unclear. Preliminary experiments have shown that it is similar to the typical response elicited by pressure-application of the $GABA_B$ agonist baclofen, and may represent the activation of $GABA_B$ receptors. The response to baclofen, like the late hyperpolarization, is a slow hyperpolarization which reverses close to the potassium equilibrium potential. The response to baclofen is typically associated with an increase in conductance. In one case where I attempted to reverse the response, it decreased in amplitude upon depolarization of the cell. The late hyperpolarization is also accompanied by an increase in conductance,

and decreases in amplitude as the cell is depolarized.

Responses to GABA and to baclofen in hippocampus parallel those in the visual cortex, and have led to the same hypothesis, that the slow, late, hyperpolarizing response to GABA and the response to baclofen may be the consequence of $GABA_B$ receptor activation (Newberry and Nicoll, 1984a and b; Inoue et al., 1985; Newberry and Nicoll, 1985). Inoue et al. have suggested that a voltage-dependent potassium channel mediates the response (Inoue et al., 1985). In area CA3 of the hippocampus, Gahwiler and Brown have shown that $GABA_B$ receptor activation opens a potassium channel (Gahwiler and Brown, 1985).

Several studies in hippocampus have revealed a second, slow phase of the IPSP (Fujita, 1979; Alger, 1984), and it has been suggested that this might be the synaptic response mediated by GABA acting on $GABA_B$ receptors (Newberry and Nicoll, 1984a and b, 1985). This may also be the case in visual cortex. The IPSP of visual cortical cells also has a second phase (Figure 8A, open arrows), and it appears to have a similar reversal potential and a similar voltage-dependence as the late hyperpolarization (Figure 8A). Thus, GABA may mediate the second phase of the IPSP in visual cortical neurons by acting at $GABA_B$ receptors.

In summary, evidence has been presented that GABA may have several functions in the visual cortex. For example, the data suggest that GABA is the transmitter of the early phase of the IPSP generated by visual cortical neurons. GABA may also be the transmitter of the late phase of the IPSP. The pattern of GABAergic innervation may serve to control the balance of excitation and inhibition of visual cortical neurons. As has been suggested by others, GABAergic neurons may also have profound effects on the processing of visual information (Pettigrew and Daniels, 1973; White, 1981; Sillito 1984; Bagust et al., 1985).

COMPARISON TO OTHER GABAERGIC SYSTEMS

In some ways the GABA responses identified for visual cortical neurons are quite similar to those of other preparations. In many species and in most areas of the CNS, perfusion or focal application of GABA elicits an inhibitory response, which is associated with an increase in conductance (Nistri and Constanti, 1979; Gallagher and Shinnick-Gallagher, 1983). This is clearly similar to the $GABA_s$ response found in rat visual cortex. It is also similar to the $GABA_d$ response, but to a lesser extent, since the effects of the $GABA_d$ response are not always inhibitory.

There are very few systems where GABA elicits bi- or multiphasic responses, or where a differential sensitivity to GABA has been shown to exist on the soma versus the dendrites of cells. This may be due to the limited number of studies which have examined responses to GABA on different areas of the same cell.

One area which is similar is the spinal cord. Three different groups have shown that GABA can hyperpolarize or depolarize these cells. First, Obata and colleagues showed that GABA depolarized and excited cultured spinal neurons of 6-day old chick embryo, but GABA hyperpolarized and inhibited cultured neurons from 10-day old embryos (Obata et al., 1978). The RMPs of these cells were approximately the same, and therefore cannot explain the results. Second, Nicoll et al. (1976) found that low concentrations of GABA hyperpolarized frog spinal motorneurons, but large doses caused depolarizations. The hyperpolarizations were inhibitory, accompanied by an increase in conductance, and were bicuculline-sensitive (Nicoll et al., 1976).

According to Barker and colleagues, when GABA is applied to the

soma of cultured cells from mouse spinal cord, hyperpolarizations are recorded, whereas when GABA is applied to dendrites, both depolarizations and hyperpolarizations occur (Barker and Ransom, 1978). This is very similar to the situation in the visual cortex. One difference is that very low concentrations (nM) of GABA actually elicited an excitatory response in the cultured spinal neurons (Barker et al., 1980). Another difference is that hyperpolarizations reversed at -64 mV, and depolarizations reversed at 0 mV (Barker and Ransom, 1978). These reversal potentials are different from those found for the $GABA_s$ (-71.7 mV) and $GABA_d$ (-49.3 mV) responses of the visual cortex, and may reflect a difference in the chloride gradients of these cells, or the ionophore itself.

In cat pericruciate cortex in vivo, Krnjevic and Schwartz (1967) found that the action of GABA on the soma of cortical neurons was similar to that of the IPSP elicited by stimulation of the cortical surface; they concluded that GABA could be a major inhibitory transmitter in the cortex. This is very similar to what we report concerning the $GABA_s$ response and the IPSP of visual cortical cells. GABA responses in rat sensorimotor cortex in vivo were also similar to those of visual cortex (Marciani et al., 1980). These authors showed that iontophoretic application of GABA produced an inhibitory response composed of a hyperpolarization and a conductance increase. Despite the similarities of these studies with ours, there were considerable differences in the calculated reversal potentials. Krnjevic and Schwartz reported a wide range of reversal potentials. The GABA responses in the study by Marciani et al. reversed near -60 mV. These differences could be attributed to application of large amounts of GABA to the soma, or to ejection of GABA in dendrites very close to the soma. In both cases the reversal

potentials would vary, or be calculated as slightly less negative than the true reversal potential of the somatic response to GABA. Differences between in vivo and in vitro preparations could also be a factor.

The GABA responses of visual cortical cells were similar in almost every respect to those reported for hippocampal pyramidal cells. I have found that GABA ejection near the soma of CA1 pyramidal cells produced a similar resonse to the $GABA_s$ response of visual cortical cells, and when applied within 200 um of the soma, a response analogous to the $GABA_{s-d}$ response or $GABA_d$ response occurred. My data are in agreement with others who have described these responses in detail (Alger and Nicoll, 1979, Andersen et al., 1980; Djorup and Mosfeldt-Larsen, 1981; Thalmann et al., 1981; Alger and Nicoll, 1982a and b). They also found that hippocampal CA1 pyramidal cells produced a hyperpolarization when GABA was applied on their cell bodies, and a combination of hyperpolarization and depolarization, or "pure" depolarizations when ejected on dendrites. The hyperpolarization reversed near the equilibrium potential of the IPSP (-70 mV in Figure 2 of Alger and Nicoll, 1982b), or -10 to -12 mV more negative than the RMP (-70 to -74 mV in Figure 8 of Andersen et al., 1980). The depolarization appeared to reverse at -40 mV (Alger and Nicoll, 1982b) or -42 mV (Djorup and Mosfeldt-Larsen, 1981). Recently another response to GABA has been characterized, which is similar to the late hyperpolarization which we have observed in visual cortical cells (Newberry and Nicoll, 1984a and b, 1985).

The similarity of cortical neurons in their responses to GABA are striking. The data suggest that the manner in which GABA controls cell activity can be very similar, despite morphological differences, differences in location in the brain, and functional role of the cell in

the neural circuitry of the region. Taken together with the work of others, it appears that responses to GABA and its mechanism of action have been at least partially conserved through evolution, since there are aspects which are similar in invertebrates and vertebrates, across vertebrate phyla (Nistri and Constanti, 1979; Gallagher and Shinnick-Gallagher, 1983), and from phylogenetically old cortex (archicortex: hippocampus) to neocortex.

PHARMACOLOGICAL CHARACTERIZATION OF GABA RESPONSES: $GABA_A$ AND $GABA_B$ RECEPTORS

1. $GABA_A$ RECEPTORS

To characterize the GABA responses pharmacologically, the effects of four different GABAergic drugs were tested on the responses to pressure-applied GABA. Each of these drugs is known to bind to a different site on the $GABA_A$ receptor complex or the $GABA_B$ receptor.

Pentobarbital enhances GABA binding to the $GABA_A$ receptor at anesthetic concentrations (100 uM; Ho et al., 1975). Therefore it was not surprising when bath-application of pentobarbital (500 uM-1 mM) greatly enhanced $GABA_d$ responses. However, it was surprising that while the $GABA_d$ response was enhanced, the $GABA_s$ response and the late hyperpolarization were depressed. The apparent decrease in amplitude of the $GABA_s$ response and late hyperpolarization might not have been a true inhibitory effect, but rather a consequence of eliciting a combination of GABA responses. The extremely large increase in the amplitude and duration of the $GABA_d$ response may have obscured the other GABA responses. Support for this possible explanation comes from dose-response studies, where increasing doses of GABA increase the $GABA_d$ response, but decrease or do not affect the $GABA_s$ response (Figure 7B1).

It has been proposed that pentobarbital may also have a direct effect on the chloride channel of the $GABA_A$ receptor (Nicoll, 1978; Olsen, 1981). The change in reversal potential of the $GABA_d$ response following bath-application of pentobarbital may reflect this effect. There also is evidence that pentobarbital depresses transmitter release through an unknown presynaptic mechanism (Nicoll et al., 1978). The presynaptic actions of pentobarbital may account for its depressive effects on orthodromic responses, such as the decrease in amplitude of the EPSP.

Bicuculline methiodide (BMI), like its parent compound bicuculline, is a competitive antagonist of GABA at the $GABA_A$ receptor (Johnston et al., 1972). It was found that BMI inhibited $GABA_s$ and $GABA_d$ responses, at concentrations that produced epileptiform activity in the slice. In two cases it depressed the late hyperpolarization.

As was the case with pentobarbital, BMI had a greater effect on the $GABA_d$ response that the $GABA_s$ response. However, BMI may have only <u>appeared</u> to be selective for $GABA_d$ responses. To explain, consider the response to GABA when it is pressure-applied near the soma. Low doses of GABA elicit a pure $GABA_s$ response, and at higher doses a $GABA_{s-d}$ response occurs. One explanation of this dose-response relationship is that when low doses are ejected GABA is applied to an area restricted to the soma. When higher doses are applied, some GABA diffuses to the dendrites to elicit the $GABA_d$ component of the response. Thus, there is a high concentration of GABA at the soma, but a low concentration in dendrites. When BMI is bath-applied, its effect will be most evident on the dendritic response to GABA, since it will be a better competitor where concentrations of GABA are lowest. Thus, BMI may affect all GABA responses equally, but appear to be selective.

These considerations make it reasonable to suggest that $GABA_A$ receptors are involved in $GABA_s$ and $GABA_d$ responses, and possibly the late hyperpolarization. However, experiments with diazepam did not necessarily support this hypothesis. On the one hand, diazepam enhanced the conductance increase of all GABA responses. This supports the hypothesis that $GABA_A$ receptors are involved in the responses to GABA. However, although diazepam enhanced the amplitudes of the $GABA_s$ response and late hyperpolarization in most experiments, it did not do so in every case. Furthermore, its effect on the $GABA_d$ response was inconsistent. Clonazepam (25 uM), which is a more potent benzodiazepine (Mohler and Richards, 1983), had no effects, and therefore did not clarify the actions of benzodiazepines on GABA responses.

There are several factors that may explain some of these inconsistencies. First, there may have been a problem regarding the dose of diazepam. Recording from cultured cells of spinal cord, MacDonald and Barker (1978) found that benzodiazepines enhance GABA responses at low doses but are inhibitory at higher doses. Gahwiler (1976) reported that the actions of GABA on cultured cerebellar neurons were inhibited by low doses of diazepam whereas higher doses enhanced the effects of GABA. Steiner and Felix (1976) have reported antagonistic actions of diazepam in cerebellum in vivo. There are only a few studies where diazepam enhanced GABA responses in cortex at all doses used (Zakusov et al., 1975; Zakusov et al., 1977; White et al., 1981).

Several laboratories have found that the effects of diazepam vary from experiment to experiment. Another study in rat cerebral cortex showed that i.v. or iontophoresis of the related benzodiazepine, chlordiazepoxide, either had no effect or antagonized the decrease in firing frequency produced by GABA (Assumpcao et al., 1979). Similar

inconsistencies have been found in hippocampus. It is useful to compare the results of the pharmacological studies in visual cortex to those performed in hippocampus, since the GABA responses themselves are so similar in these two areas. Alger and Nicoll found that diazepam enhanced the hyperpolarization produced by somatic application of GABA, but only in 3 of 6 cells (Alger and Nicoll, 1982b), and in the cells that demonstrated effects, the changes were small. This is similar to what has been found in visual cortex. In other respects as well, the pharmacological sensitivity of GABA responses in visual cortex was similar to that in hippocampus. For example, Alger and Nicoll found that both pentobarbital and bicuculline had the greatest effects on dendritic responses to GABA, which are analogous to the $GABA_d$ response in visual cortex (Alger and Nicoll, 1982b).

Another factor to consider concerning the effects of diazepam is the reported heterogeneity of benzodiazepine receptors, which has been used to explain inconsistencies in some of the binding studies using benzodiazepines (Squires et al., 1979; Haefly et al., 1983; Mohler and Richards, 1983). It appears that only some receptors for benzodiazepines are linked to the $GABA_A$ receptor (Seighart and Karobath, 1980; Unnerstall et al., 1981). If this is the case, then in visual cortex some of the neurons may have receptors for benzodiazepines, whereas others may not. Another possibility is that receptors may exist only on cell bodies or only on dendrites. In fact, there is some evidence that benzodiazepine receptors are unevenly distributed in the cortex. Young and Kuhar (1980) demonstrated that binding sites for benzodiazepines in the cerebral cortex are concentrated in Layer IV. One last suggestion is that only one of the actions of diazepam might involve GABA receptors, and there is evidence for this. It has been proposed that benzodiazepines may enhance

the depressant actions of adenosine by preventing its reuptake (Phillis, 1975). There is also some evidence that benzodiazepines may affect a calcium-dependent potassium current (Carlen et al., 1983).

In conclusion, there is evidence that the $GABA_A$ receptor is involved in $GABA_s$ responses and $GABA_d$ responses. Although some of the data suggest that $GABA_A$ receptors mediate the late hyperpolarization, another hypothesis is offerred below, which postulates that $GABA_B$ receptor activation is responsible for the late hyperpolarization.

2. $GABA_B$ RECEPTORS

The $GABA_B$ agonist baclofen did not affect responses to pressure-applied GABA. This may be because $GABA_B$ receptors were already maximally activated by pressure-applied GABA, or because the pressure pipette was not located in the vicinity of $GABA_B$ receptors, so regardless of the concentration of agonist, an effect would not be apparent.

However, baclofen did have an effect by itself. When bath- (100 uM) or pressure-applied (100 uM- 1 mM), it hyperpolarized the membrane potential and decreased the R_{in}. These effects appeared to reflect actions on postsynaptic membrane, since they were unaltered when synaptic transmission was blocked by tetrodotoxin.

When relatively low doses of baclofen (1-10 uM) were used to fill the pressure pipette, typically a hyperpolarization occurred without a decrease in input resistance. This suggested that the change in RMP and R_{in} may be due to two processes, and that the change in RMP is more sensitive to baclofen than the change in R_{in}. Further support for this hypothesis came from analyzing the time course of recovery of the typical response to higher concentrations of baclofen; the RMP took longer than the R_{in} to return to control levels.

These effects were identical to those observed in hippocampus (Newberry and Nicoll, 1984a and b, 1985), and together they are the first cases where a postsynaptic site of action for baclofen has been identified. In other studies, most of which were performed in ganglia or in cultured cells, baclofen is thought to act presynaptically, where it decreases calcium influx and thus transmitter release (Bowery et al., 1980; Dunlap and Fischbach, 1981; Bowery et al., 1983). Baclofen may have had a presynaptic effect in visual cortex as well. When tetrodotoxin was not used, EPSPs were greatly reduced in amplitude during bath application of baclofen. However, the decrease in R_{in} might also have caused this to occur, so it is not certain whether the effect was presynaptic.

The effects of baclofen provided a clue to the possible identity of the late hyperpolarization, because there were several similarities between the late hyperpolarization and the response to baclofen. For example, both consist of a long-lasting hyperpolarization accompanied by an increase in conductance. Both can be elicited following ejection of agonist (GABA or baclofen) on the soma or the dendrites. Finally, there is evidence that potassium ions are involved in both responses. Both appear to reverse near the equilibrium potential for potassium (approximately -80 mV, late hyperpolarization; -90 mV, response to baclofen). There is evidence that a similar response to baclofen in hippocampus is potassium-mediated, and my data suggest that the late hyperpolarization is potassium-dependent. It is possible that the late hyperpolarization reflects the action of GABA at $GABA_B$ receptors, and that these receptors are linked to potassium channels. This hypothesis is supported by the fact that the $GABA_A$ ligands BMI and pentobarbital did not have dramatic effects on the late hyperpolarization. Although the

$GABA_A$ ligand diazepam did potentiate the late hyperpolarization greatly, this may have been through an action of diazepam that does not involve the $GABA_A$ receptor. It has been previously discussed that diazepam may, in fact, have other actions, one of which may involve a potassium channel.

IONIC MECHANISMS OF GABA RESPONSES

1. CHLORIDE

It has been known for some time that the $GABA_A$ receptor surrounds a chloride channel (Nistri and Constanti, 1979; Olsen, 1981) Therefore it was not surprising to find that $GABA_s$ and $GABA_d$ responses were affected by the use of recording electrodes filled with a high concentration of chloride.

It is unclear why only some of the $GABA_d$ responses were affected by chloride. One explanation is based on the fact that $GABA_d$ responses may be generated in dendrites. Since the recording electrode was in the soma, the high chloride concentration may have only affected somatic areas. There are several reasons why chloride might not have reached the area of the dendrite where $GABA_d$ responses were being generated. First, diffusion might not be an effective means of raising the intracellular chloride concentration. It may be necessary to eject chloride ions by using hyperpolarizing current. Very little hyperpolarizing current was given while recording from these cells. Second, the diffusion from the recording electrode into the cell might have be hindered, if the electrode tip were abutting on membrane or subcellular organelles, such as mitochondria. It is estimated that the diameter of intracellular electrodes at their tips are a few microns at the most, so this

possibility is feasible. Diffusion would also be difficult if the response was generated on dendrites which appear to be proximal, but actually wrap around the soma or bend back on themselves, so that they are actually quite far from the soma. In this case, chloride would have to travel a long distance to get to the site where the GABA response is generated.

It appears that the late hyperpolarization was not altered by the use of electrodes filled with KCl. The reversal potential for this response did not change, which is an indication that the response was not affected. Although the response was only observed at three of the sites of ejection in the cells examined, this does not necessarily indicate that it was affected by use of potassium chloride-filled electrodes, since even with potassium methylsulphate-filled electrodes the response was not observed at all sites of ejection. Thus, chloride appears to mediate the $GABA_s$ and the $GABA_d$ responses, but not the late hyperpolarization.

2. POTASSIUM

When the extracellular concentration of potassium was tripled, alterations of all three types of GABA responses were apparent. First, the $GABA_s$ response reversed polarity and its reversal potential became less negative. The $GABA_d$ response increased in amplitude and its reversal potential became less negative also. Finally, in the one cell where it was present in control, the late hyperpolarization reversed polarity and demonstrated a large change in reversal potential.

The data suggest that there is a potassium component to all three GABA responses. The strongest evidence is for a role of potassium in the late hyperpolarization, which showed a Nernstian dependence on potassium

and was unaffected by other ions. In contrast, there are several reasons to be apprehensive of attributing $GABA_s$ and $GABA_d$ responses to potassium ions. First, the data using KCl-filled electrodes suggests a role of chloride ions in these responses. Second, the changes in reversal potentials of these responses following bath-application of high potassium were not always Nernstian (Katz, 1966; Ferriera and Marshall, 1985), and therefore these responses probably involve other ions besides potassium. Third, changes in extracellular potassium concentration can modify the gradient for chloride (Dseiz and Lux, 1982), and therefore indirectly affect GABA responses via an effect on chloride ions. This has been proposed to account for the change in the reversal potential of the GABA response following bath-application of high potassium in thalamic reticular neurons recorded in thalamic slices (McCormick and Prince, 1986). This could account for the relatively small changes in reversal potential of the $GABA_s$ and $GABA_d$ responses, but not the dramatic change in the late hyperpolarization.

Taking these considerations into account, $GABA_s$ and $GABA_d$ responses are probably mediated by chloride and, to some extent, potassium. In contrast, the late hyperpolarization appears to be only influenced by potassium.

3. CALCIUM

To examine the involvement of calcium, Krebs-Ringer solution containing 0 mM calcium chloride and 10 mM magnesium chloride was bath-applied. In all cases synaptic transmission was depressed or blocked. The $GABA_s$ and $GABA_d$ responses were slightly decreased (n=1) or were unaffected (n=3), and in no case were there effects on the reversal potentials of either response. Another approach was taken in a fourth

experiment, where the calcium antagonist manganese was used. In this case Krebs-Ringer solution, which contained manganese chloride substituted in equimolar concentrations for calcium chloride, was bath-applied. In this experiment, there was no effect of the altered buffer solution on the responses to GABA.

There is still a possibility that calcium may influence GABA responses, because some calcium-dependent events, such as the AHP following depolarizing current pulses (Barrett and Barrett, 1976; Krnjevic et al., 1978), were not blocked in these experiments. However, the results suggest that calcium ions do not contribute to the responses to GABA.

4. SODIUM

When the sodium channel blocker tetrodotoxin (TTX) was bath-applied, responses to GABA were unaltered. There is a possibility that synaptic sodium ions could contribute to some of the actions of GABA, because most synaptic sodium channels are not affected by TTX. However, the results suggest that sodium ions do not mediate the responses to GABA.

The lack of effects of the experiments using low calcium, calcium channel blockers, or sodium channel blockers, supports the hypothesis that $GABA_s$ and $GABA_d$ responses are mediated by a combination of potassium and chloride ions, and that the late hyperpolarization is mediated solely by potassium ions.

CONCLUDING STATEMENT

In this study, the responses to GABA of Layer V rat visual

cortical neurons have been identified. One of the most important findings was that all cells tested responded to GABA applied within 300 um of the soma, regardless of the morphology of the cell. Three responses to GABA could be elicited. The $GABA_s$ response was elicited primarily following somatic ejections of GABA and reversed near the RMP. It probably reflects the actions of inhibitory GABAergic interneurons, which are known to cover pyramidal cell bodies and initial axon segments with synapses. Upon binding of GABA to a $GABA_A$ receptor, a chloride-dependent IPSP would be produced and serve to strongly inhibit pyramidal cell activity. Two questions remain unanswered: 1) are these responses solely generated on somatic membrane, and 2) do potassium ions play a role in this response? If so, the receptor involved must be different from the classical $GABA_A$ receptor, since the $GABA_A$ receptor is coupled to a chloride channel. Future experiments, such as the bath-application of Krebs-Ringer buffer containing a low concentration of chloride, could help answer this question, since the results of such experiments could determine whether the chloride-dependence of the $GABA_s$ response is Nernstian.

The $GABA_d$ response was elicited by dendritic application of GABA, and reversed approximately 20 mV positive to the RMP. The $GABA_d$ response could be quite important, since its depolarizing action might have excitatory influences, whereas the conductance increase accompanying the response might be inhibitory. It may underly the fine tuning of responses to visual stimuli, which has been known for some time to be strongly influenced by GABAergic drugs (Curtis and Felix, 1971; Pettigrew and Daniels, 1973; Rose and Blakemore, 1974; Daniels and Pettigrew, 1975; Sillito, 1975a and b; Bagust et al., 1985). Pharmacological data strongly suggested that the actions of the $GABA_d$ response were mediated by $GABA_A$ receptors. In contrast, its ionic dependence on chloride ions was less

clear, perhaps due to the difficulties of recording a dendritic event with a somatic electrode.

The late hyperpolarization was a long-lasting response that kept the cell at potentials negative to the RMP. Since it was not elicited in all cells, was of small amplitude, and appeared to be voltage-dependent, this response remains a mystery to some extent. However, it also has been illuminating, in that it is one of first examples of a postsynaptic action of the recently identified $GABA_B$ receptor. Ion substitution experiments suggested that this receptor is coupled to a potassium channel. There is some evidence, in fact, that the $GABA_B$ receptor is coupled to a potassium channel (Gahwiler and Brown, 1985; Inoue et al., 1985). It may be that the potassium-dependence of the $GABA_s$ and $GABA_d$ responses actually reflects the potassium-dependence of the late hyperpolarization. This might occur if the late hyperpolarization actually has an early onset, and therefore can contaminate $GABA_s$ and $GABA_d$ responses. Use of compounds such as bicuculline to block the $GABA_s$ and $GABA_d$ responses, might be a future approach to further investigate the "late" hyperpolarization.

Finally, these observations may be of clinical use in the approaches to treatment of several CNS disorders thought to be caused by dysfunctional inhibitory systems. The effects of pharmacological intervention with a GABAergic agent can perhaps be better predicted by knowing more about how GABA affects cortical neurons. Use of GABAergic compounds will undoubtedly influence many different cortical systems, and may affect them in very similar ways.

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